Anti-diarrhoeal Activity of *Hydnora abyssinica* Aqueous Root Extract in Rats

By

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To you all …

Who

always love me,

always be pleasure of my success

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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First of all, my thanks and praise to almighty Allah, The Beneficent, The Merciful, for giving me health and strength to accomplish this work. Then I would like to express my sincere appreciation and deep gratitude to my supervisor, Dr. Samia Mohammed Ali El Badwi, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Khartoum for her appropriate guidance, encouragement and patience.

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ABASTRACT

This study was designed to investigate the anti-diarrhoeal activity of the orally administrated extract of *Hydnora abyssinica* roots at different doses to rats. The anti-diarrhoeal potential of plant extract was tested in rats by faecal mass inhibition. The toxicity of the plant extract was also tested at different doses to rats via oral route.

The aqueous extract of *Hydnora abyssinica* was tested using twenty- four Albino rats arranged in five groups (control+ 3 test groups + loperamide group) receiving oral dose rates of 100, 200 and 400 mg extract/kg body weight/rat, 3 mg loperamide (reference anti-diarrhoeal drug)/kg body weight/rat and 1 ml/kg body weight/rat of normal saline (control). All individuals were given orally, 30 minutes subsequent to extract administration, acute diarrhoea inducer (1 ml castor oil /kg body weight/rat). Cumulative wet faecal mass was weighed for assessment of diarrhoea state at 4 and 6 hours.

The safety of the extract was also tested, using twenty Albino rats distributed in four groups (control + 3 test groups) given oral dose of 400, 800 and 1600 mg extract/kg body weight/rat/day, continued for seven days.

The aqueous extract of *Hydnora abyssinica* showed significant ($P<0.05$) anti-diarrhoeal activity against castor oil induced diarrhoea in all rats with reduction rates of 46.78%, 63.21% and 74.68% for the doses 100, 200 and 400 mg/kg body weight respectively at 4^{th} hrs post treatment and these doses rates recorded inhibition rates of 27.42%, 50.88% and 60.13% at 6^{th} hours respectively post treatment.

Clinical signs were observed regularly. Sera were analyzed for enzymatic activities of AST, ALT and metabolic indicators such as total protein, albumin, sodium, potassium and calcium. Also, changes in values

of RBC_S, Hb, PCV, MCV and MCHC were recorded. The weight of faeces was recorded too for assessment of diarrhoeal inhibition.

In both experiments, clinically no signs of toxicity were observed, no deaths or gross changes in the examined vital organs.

Metabolic indicators; total protein, potassium and calcium demonstrated significant increase while sodium and albumin showed no significant changes at dose 400 mg/kg.

In haematological parameters there were significant decrease in values of RBC_s, PCV and MCHC while Hb concentration showed significant increase. MCV values indicated no changes.

Plant extract's safety showed no significant change in the serum (ALT) activity while in (AST) activity there was significant increase. In serum metabolites, there were no changes in the concentration of total protein and albumin when compared with the un-dosed control group at the end of the experiment. The concentration of sodium and potassium showed significant increase.

There were significant decrease in the concentration of haematological parameters tested; RBC_S, Hb and PCV. MCHC showed significant increase, while MCV values demonstrated no changes.

The results were discussed with suggestions for future studies.

بسم الله الرحمن الرحيم

المستخلص

صممت هذه الدراسة لإختبار قدرة تضاد الإسهال للمستخلص المائى لنبات الترتوس، بجرعات مختلفة، فى الفئران عبر التجريع الفموى وذلك بقياس نسبة التثبيط الناتج فى وزن البراز الكلى. إختبرت أيضاً سمية المستخلص المائى للنبات فى الفئران عن طريق الفم.

إختبرت فعالية تضاد الإسهال للمستخلص المائى لنبات الترتوس باستخدام 24 فأراً أبيضاً فى خمس مجموعات (مجموعة سيطرة + 3 مجموعات إختبار+ مجموعة اللوبراميد) أعطيت فموياً الجرعات 100، 200 و400 ملجرام/كجم وزن حى /فأر، 3 ملجرام لوبراميد (دواء مرجع تضاد إسهالات)/كجم وزن حى/فأر و1مل/كجم/وزن حى/فأر من المحلول العادى لملح الطعام (مجموعة السيطرة). قبل 30 دقيقة من إعطاء مستخلص النبات تم تجريع جميع الفئران بمسبب إسهال موضعى (1 مل/كجم وزن حى/فأر زيت خروع). تم وزن البراز الناتج بعد أربع ساعات وست ساعات وأربع وعشرون ساعة لتقييم فعالية المستخلص فى تضاد الإسهال.

تم أيضاً إختبار سمية المستخلص المائى لنبات الترتوس باستخدام 20 فأراً أبيضاً فى أربع مجموعات (مجموعة سيطرة + 3 مجموعات إختبار) 400، 800 و1600 ملجرام/كجم وزن حى/فأر/اليوم ولمدة 7 أيام.

أظهر المستخلص المائى لنبات الترتوس فعالية معنوية (أ<0.05) فى قدرة تضاد الإسهال الناتج والمتسبب بزيت الخروع بتثبيط وزن البراز الناتج وذلك فى جميع الجرعات 100، 200 و400 ملجرام/كجم وزن حى/فأرفى الساعة الرابعة للرصد بعد المعالجة معطياً نسبة تناقص 46.71%، 63.21% و74.68% على التوالى وفى الساعة السادسة للرصد بعد المعالجة أظهر تناقصاً معنوياً بنسبة 27.42%, 50.58% و60.13% على التوالى. كل الفئران المتبقية ذبحت فى نهاية التجربة لملاحظة التغير فى الأعضاء الحيوية. تمت مراقبة الأعراض

الإكلينيكية بأنتظام. تم تحليل المصل لكشف تركيز ناقل القلوتاميت أوكساليت وناقل إيمين القلوتاميت بيروفيت وكذلك قيم المؤشرات الأيضية: البروتين الكلى، الألبيومين، الصوديوم، البوتاسيوم والكالسيوم. سجلت كذلك التغيرات فى عدد الكريات الحمراء، تركيز الهيموجلوبين، حجم الخلايا المتراص، متوسط حجم الخلايا ومتوسط تركيز الهيموجلوبين للخلية.

فى كلتا التجربتين إكلينيكياً لم تلاحظ أى علامات تسمم، موت أو تغيرات مرئية فى الأعضاء الحيوية التى تمت معاينتها.

قيم المؤشرات الأيضية فى البروتين الكلى، البوتاسيوم والكالسيوم أظهرت زيادة معنوية بينما قيم الصوديوم و الألبيومين لم تظهر أى تغيير معنوى أما الصورة الدموية أظهرت نقصان فى عدد الكريات الحمراء، حجم الخلايا المتراص ومتوسط تركيز الهيموجلوبين للخلية بينما أظهر تركيز الهيموجلوبين زيادة معنوية أما متوسط حجم الخلايا لم يظهر أى تغيير معنوى .

عند إختبار سمية المستخلص المائى لنبات الترتوس لم توجد تغيرات معنوية فى نشاط تركيز ناقل القلوتاميت أوكساليت فى المصل أما نشاط ناقل إيمين القلوتاميت بيروفيت أظهر زيادة معنوية. تحليل المصل أظهر عدم وجود تغيير فى تركيز البروتين الكلى و الألبيومين مقارنة مع مجموعة السيطرة. من جهة أخرى هناك زيادة معنوية فى تركيز الصوديوم و البوتاسيوم. أما الصورة الدموية أظهرت نقصان معنوى فى عدد الكريات الحمراء، تركيز الهيموجلوبين، حجم الخلايا المتراص، أما متوسط تركيز الهيموجلوبين للخلية أظهر زيادة معنوية بينما متوسط حجم الخلايا لم يظهر أى تغيير معنوى.

تمت مناقشة المناحى التطبيقية والمقترحات المستقبلية للدراسة.

INTRODUCTION

Plants have been used for thousands years for many different purposes ranging from medicinal to religious (in rituals) and from protection against spirits to culinary delights, perfumery and cosmetics (EL Ghazali *et al*., 2004). They contain a number of chemical constituents such as alkaloids, flavonoids, tannins, saponins, glycosides and others, which have been isolated and used as an important source of indispensable drugs. Clarke *et al*. (1981) stated that medicinal plants are known by their required clinical effects on the abnormal living tissues or organs while toxic ones are known by their ability to cause a non required physiological deviation in animals' bodies.

However, medicinal plants are now being given a serious attention as is evidenced in 1970 by the recommendation given by the World Health Organization (WHO), that proven traditional remedies should be incorporated within national drug policies (Wondergen *et al.*,1989) and since 1976, the traditional medicine programme of (WHO) as a source of Primary Health Care (PHC) has been globally addressed (Rukangira, 2001).

A list of 20.000 medicinal plants used worldwide is prepared by the (WHO) and indicated that 4.000 plant origin drugs are used in a wide range through out the world thus, recognition and development of medical and economic benefits of traditional medicinal plants are on increase in both developing and industrialized countries (WHO, 1998). In a country like China, with its all this population, plants extracts which have been used for centuries, are still the main type of drug therapy. A survey showed that there are 7295 species of plants used medicinally in China (Ciba Found, 1994).

In East African, Koko Waro, (1976) reported that both literate and illiterate people still use local plants as drugs in many conditions. Plants were also traditionally prescribed and used for generations and centuries with slight or almost no changes and with strong beliefs in North Africa, leading in most cases to satisfactory results (Boulos, 1983).

Sudan, the largest country in Africa, has a wide diversity climate which is responsible for its varied vegetation and very rich flora. Many species of plants grow abundantly in the Sudan and other African countries and are used by the village populations for treatment of various disorders (Adam, 1978 and Nwude,1979).

The Sudanese folkloric medicine represents a unique blend of indigenous cultures with Egyptian, Indian, Arabian, East and West African cultures (Gamal *et al.*, 1997). However, it is of paramount importance that people remain aware of the dangers caused by plants which are utilized for medicinal purposes and need to validate their safety, this lead researchers to study more about these plants to determine their mode of action and pharmaceutical values. However, the results of many researches in Sudan demonstrated potentials for control of pests, schistosomiasis, fascioliasis, malaria, diabetes and other important diseases.

One of the most serious health problems in both man and animals is diarrhoea which occurs in all age groups, especially in the young. In new born farm animals, diarrhoea is one of the most common disease complexes. Diarrhoea also is one of the most commonly observed abnormalities in faecal consistency and frequency of defecation (Radostits *et al.*, 2000). It is a significant cause of economic loss especially in cattle herds and may assume even greater importance as livestock becomes more intensified. The economic loss is due not only to

mortalities, but also to medical costs and poor growth (Woode and Crouch, 1978).

Snodgrass (1981) reported that 73% of neonatal mortalities of calves in a Libyan farm were caused by neonatal calves' diarrhoea. In a Sudanese diary herd of local zebu breads belonging to the University of Khartoum, Wakeen and Dansoury (1962) reported a calf mortality rate of 19% during the period 1944 to 1961, 70.3 % of the total deaths were chiefly due to scours and enteritis.

Diarrhoeal diseases are also identified as the largest single cause of death among children in the developing countries (Cruickshank, 1973). Indeed diarrhoea produces more illness and causes death of more infants and children than all other diseases combined (Weber, 1976) and still diarrhoea is one of the leading causes of morbidity and mortality of children less than 5 years worldwide, causing about one billion episodes of illness and 3−5 million deaths annually (WHO, 1980 and Behrman *et al*., 2004). To combat the problem of diarrhoea in developing countries, the world health organization (WHO) has constituted a diarrhoea disease control programme (DDC) which includes studies of traditional medicine practices together with the evaluation of health education and prevention approaches (Syder and Merson, 1982).

The two main dangers of diarrhoea are death and malnutrition. Death from acute diarrhoea is commonly due to dehydration. Another important cause of death is dysentery which causes damage to the intestine by systemic infection and malnutrition (WHO, 1992).

It is important to note that many of the organisms which cause scours in livestock can cause disease in humans (Schoenian, 2009). Hence calves scour and diarrhoeal diseases in children caused by various agents including *E. coli* are becoming a health problem worldwide including Sudan (Lambert, 1979 and Puent and Finlay, 2001).

In Sudan, the mortality rate among children was estimated as 9% and more than half of this percentage was due to diarrhoeal diseases (Abd El Gadir, 2004) while mortality rate among calves was estimated to be 10% and in some months it may reach 100% due to diarrhoea (Ali, 2000).

Despite the emergence of a number of drugs, none has found a place in the successful management of diarrhoea (Farthing, 2002). Therefore, there is need for continuous search for drugs that might inhibit the process of diarrhoea development. Rani *et al*. (1999) stated that medicinal plants are promising a source of anti-diarrhoeal drugs. Thus medicinal plants are one of the useful areas of research in this regard and which are believed to possess anti-diarrhoeal activities, are used in most of the developing countries in the management of diarrhoea.

The popular medicinal plant *Hydnora abyssinica*, which is known locally as (Tartous), has grown abundantly in the tropical regions as well as in Sudan. The infusion of the roots powder is traditionally used in folkloric medicine for various disorders especially in treatment of diarrhoea and dysentery. However, this has not been confirmed and no previous scientific literature was found on its anti-diarrhoeal activity. Hence, the present study has been designed to investigate the anti-diarrhoeal activity of the roots extract of *Hydnora abyssinica* in experimentally-induced diarrhoea model in rats and to test its toxicity to rats.

The objectives of the study are to:

- Evaluate the anti-diarrhoeal potential of the aqueous extract of *Hydnora abyssinica* (*Tartous*) roots against castor oil induced diarrhoea in rats.
- Investigate the safety of *Hydnora abyssinica* roots aqueous extract when administrated orally to rats at different doses.

CHAPTER ONE

LITRITURE REVEW

1. 1 Definition of diarrhoea

In medicine diarrhea (from the Greek, ″diarrhoia″ meaning ″a flowing through″, Medterms dictionary, 2007), also spelled diarrhoea, is the condition of having frequent loose or liquid bowel movements.

According to Heywarth, (1985); Martin Richard, (1989); Radostits *et al.*, (2000) and Schoenian, (2009) diarrhoea is the increased frequency of defecation accompanied by faeces which contain an increased concentration of water and decrease in dry matter content. The volume of faecal excretion is also, increased. The consistency of the faeces varies from soft to liquid. They may contain blood or mucous, be smelly and the colour may be abnormal.

Abnormalities of peristalsis and segmentation usually occur together and when there is a general increase in peristaltic activity, there is increased caudal flow resulting in a decrease in intestinal transit time and diarrhoea. Because of a lack of absorption of fluid the faeces are usually softer than normal, the dry matter content is below the normal range, and the total amount of faeces passed per day is increased. The frequency of defecation is usually also increased.

However, it is difficult to define diarrhoea exactly, since there is great individual variation in normal stool frequency.

1. 2 Types of diarrhoea

According to duration and episode, three clinical syndromes of diarrhoea have been defined, each reflecting pathogenesis and requiring different approaches to treatment (WHO, 1992); these include:

1. 2. 1 Acute watery diarrhoea

It begins acutely for less than 14 days and the passage characterized by loose or watery stools without visible blood. Vomiting may occur and fever may be present (WHO, 1992). Acute diarrhoea usually happens due to faecal oral transmission of bacterial toxins, viruses and bacteria or protozoan organisms (Kasper *et al.*, 2005).

1. 2 .2 Dysentery

This is diarrhoea with visible blood in the faeces. Important effects of dysentery include anorexia, rapid weight loss and damage to the intestinal mucosa by invasive bacteria (WHO, 1992).

1. 2. 3 Persistent diarrhoea

This diarrhoea begins acutely, but of unusually long duration (at least 14 days). The episode may begin either watery diarrhoea or as dysentery. Marked weight loss is frequent. There is no single microbial cause for persistent diarrhoea (WHO, 1992).

1. 3 CAUSES

There are many causes of diarrhoea in farm animals and depending upon the causative agent diarrhoea varies considerably in its severity. Most workers agreed with the observation that numerous agents were involved in the diarrhoea syndrome (Fernelius, 1973). Studies of Radostits *et al.*, (2000) showed that several infectious agents, environmental, nutritional, immunological and genetic factors were involved in precipitating this disease.

1. 3. 1 Enteritis

The term enteritis is used to describe inflammation of the intestinal mucosa resulting in diarrhoea, sometimes dysentery, abdominal pain occasionally, varying degrees of dehydration and acid base imbalance, depending on the causes of the lesion, its severity and location. In many cases, gastritis also occur together with enteritis.

The enteropathogens most commonly found and extensively studied were:

1. 3. 1. 1 Bacteria

Enterotoxgenic *Esherichia coli* (ETEC) in newborns (Tzipori *et al.*, 1984) causes outbreaks commonly and usually colostral immune status determine survival, *Salmonella spp.* affect all ages; stress induced causing outbreaks (Radostits *et al.*, 2000) and *clostridium perfringes* (Dickie *et al.,* 1978) cause severely diarrhoea in different species; are the most commonly bacterial causes of diarrhoea while *Mycobacterium paratuberculosis, Protueus spp., Pseudomonas spp., Actinobacillus equi* and *Treponema hyodysenteriae* (swine dysentery) are other causes of diarrhoea in specific species.

1. 3. 1. 2 Viruses

Rotavirus and coronavirus are considered to be the most important causes of viral diarrhoea in new born farm animals (Blood *et al.* , 1983; Janke, 1989) which result in explosive outbreaks. Transmissible gastroenteritis virus TGE causes severe outbreaks in piglets (Radostits *et al*., 2000), bovine viral diarrhoea virus BVDV (mucosal disease virus MDV) in adult cattle (Lambert and Fernelius, 1968; Mebus, 1976), Malignant catarrhal virus and Rinder pest virus can also cause diarrhoea.

1. 3. 1. 3 Fungi

Candida spp. causes diarrhoea in young animals following prolonged use of oral antibacterial (Radostits *et al*., 2000).

1. 3. 1. 4 Helminthes

Variety of helminthes such as *Ostertagia spp., Nematodirus spp., Trichostrongylus spp., Trichonema spp., Strongylus spp., Ascaris spp.* and *Trichuris suis* result in diarrhoea in different animals' species.

1. 3. 1. 5 Protozoa

Cryptosporidium spp. and *Eimeria spp.* are extracellular parasite that commonly causing diarrhoea (Pohlenz et al., 1978).

1. 3. 2 Chemical agents

Arsenic, fluorine, copper, sodium, chloride, mercury, molybdenum, nitrates, poisonous plants and mycotoxins in all ages with history of access to substances, diarrhoea outbreaks occur (Radostits *et al.*, 2000).

1. 3. 3 Physical agents

Sand, soil, silage and feed containing lactic acid (sour brewers' grains) usually in mature cattle with history of access, cause diarrhoea outbreaks (Blood *et al.* , 1983).

1. 3. 4 Nutritional deficiencies

Copper deficiency conditioned by excess molybdenum usually in mature cattle on pasture with high levels of molybdenum, also iron deficiency in piglets result in diarrhoea (Radostits *et al.*, 2000).

1. 3. 5 Dietary

Most common in the neonatal animals when over fed on milk or with indigestion which usually occurs following a sudden change of diet particularly in animals at weaning time, in mature cows occur usually when move from dry pasture to lush pasture or when grains are added liberally to feedlot at first time.

Because of the relatively high cost of good quality skim milk powder, large quantities of both non milk proteins and carbohydrates are used in formulating milk replacers, while some calves in the large units can satisfactory digest the nutrients in these milk replacers, many cannot and this leads to high incidence of diarrhoea, secondary colibacillosis and enteric salmonellosis, loss of weight, emaciation and starvation (Radostits *et al.*, 2000), so the use of large quantities of soybean protein and fish

protein concentration in milk replacer for calves will result in chronic diarrhoea and poor growth rates.

1. 3. 6 Malabsorption

This is due to villous atrophy and in hypocuprosis (due to molybdenum excess). Malabsorption syndromes are being recognized with increased frequency in monogastric farm animals. In malabsorption there is usually diarrhoea, always failure to grow or maintain body weight, in spite of an apparently normal appetite and adequate diet.

Woode and Crouch, (1978) stated that during the stage of diarrhoea, the absorption was reduced 60 to 90%.

The location of the lesion in the intestinal tract may influence the severity of the enteritis or malabsorption. Lesions involving the small intestine are considered to be more acute and severe than those in the large intestine because approximately 75−80% of the intestinal fluids is absorbed by the small intestine and much lesser quantities by the large intestine. However, the horse is an exception; 95% of these fluids are reabsorbed by the large intestine. Studies of Radostits *et al*., (2000) showed that any significant dysfunction of the absorptive mechanism of the large intestine of the horse would results in large losses of fluids and electrolyte. This may explain the rapid dehydration and circulatory collapse which occur in horses with colitis−X.

1. 3. 7 Miscellaneous or Uncertain etiology

Radostits *et al*., (2000) reported more other causes that result in diarrhoea:

- Excitements when take place leads to neurogenic diarrhoea.
- Local structural lesion of the stomach or intestine including; ulcer of the abomasum or stomach, and tumer as intestinal adenocarcinoma.
- Carbohydrate engorgement in cattle.

- In some cases of ileal hypertrophy, ileitis, diverticulitis and adenocarcimoma.
- Terminal stages of congestive heart failure (visceral edema) or hepatic fibrosis.
- Endotoxic mastitis in cattle (splanchnic congestion).
- Vagus indigestion in cows causes pasty faeces but bulk is reduced.

These cases may be mistaken initially for other causes of diarrhoea.

Morin *et al*., (1978); Tzipori, (1981) and Radostits *et al*., (2000) stated that diarrhoea can be attributed to infection with single or multiple agents. As diagnostic techniques are improved, multiple mixed infections are being recognized more frequently than single infection.

There appear to be some synergistic action between *E. coli* and viruses in the production of the typical infectious calf diarrhoeal disease (Amstutz, 1965). In experimental combined *E. coli* and rotavirus infection in lambs, the mortality is higher than when either of the two agents administered alone (Blood *et al.*, 1983). Baljer, (1986) suggested that more severe forms of diarrhoea are usually the result of mixed infections and recent research has identified a synergic effect between rotaviruses and enterotoxigenic *E.coli*.

1. 4 Epidemiology and Predisposing agents

According to Quinin *et al*., (1999) the predisposing causes are of paramount importance and to a large extent determine weather or not clinical signs of illness will occur.

Mebus, (1976) and Radostits *et al*., (2000) reported that in addition to the primary etiological agents of diarrhoea, there are many influences exerted by the host and the environment which can play an important role in facilitating or suppressing the ability of the causative agent to cause diarrhoea. Thus, deficiency in colostral immunoglobulins in the newborn calves and piglets make them more susceptible to diarrhoea, and with a

high mortality rate, than animals with adequate levels. The stresses of transportation or deprivation of feed and water are commonly precipitate enteric salmonellosis. Also the stress of weaning in pigs is a risk factor for post weaning diarrhoea, while the prolonged orally use of antimicrobials in all species may alter the intestinal microflora and allow the development of a superinfection by organisms which would not normally cause disease. Blood *et al*., (1983) stated that over crowding of the dam and calf rearers are other epidemiological factors that caused diarrhoea in newborn calves. Woode and Dennis, (1978) found that calves born to heifers have higher incidence of the disease and are most seriously affected and hence high morbidity and mortality. A study of Woode and Crouch, (1978) found that, under experimental conditions, a drop of 10−20º C of the ambient temperature increased the mortality associated with enteric pathogens.

In North America, Babuik *et al.*, (1985) reported that the clinical disease is prevalent during the calving season in late winter or early spring while there is no evidence of infection for the other 8−10 months. Ferris (1971) also, reported that although coronavirus induced disease can occur through out the year, peak incidence occurs in early spring were calving is often concentrated. Prevalence studies have found that about 70% of cryptosporidiosis cases occur also during winter (Anderson and Hall, 1985).

Babuik *et al*., (1985) suggested that the two possible causes of emergence or disappearance of the disease are due to the virus which may persist in the environment protected by organic matter and the small number of adults that are persistently infected showing no clinical signs but shedding the virus, he concluded to that this stability of most enteric viruses in organic matter and large numbers of virus particles shed in faeces (10^{11} rotavirus particles/g of faeces) ensures the environmental

contamination and infection. Rhodes *et al*., (1979) showed that with electron microscopy, the virus can be detected for up to 6−10 days after the onset of diarrhoea.

1. 5 Pathogenesis

1. 5. 1 Mechanisms of diarrhoea

Under normal condition, a large quantity of fluid enters the small intestine from the saliva, stomach, pancreas, liver and intestinal mucosa. This fluid and its electrolytes and other nutrients must be absorbed mainly by the small intestine, although large quantities move into the large intestine for digestion and absorption, especially in the horse. Any dysfunction of the intestine will result in failure of adequate absorption and diarrhoea (Radostits *et al*., 2000), depending on the causative agent, he concluded to that intestinal malabsorption may be the result of at least four different mechanisms:

1. 5. 1. 1 Osmotic diarrhoea

There is an osmotic effect when substances within the lumen of the intestine increase the osmotic pressure over a greater than normal length of intestine, resulting in an osmotic movement of an excessive amount of fluid into the lumen of the intestine. The fluid is not reabsorbed and accumulates into the lumen. Saline purgatives, overfeeding, indigestible feeds and disaccharidase deficiencies are some of these substances.

The usual pathogenetic sequence of events is selective destruction of villous absorptive cells, villous atrophy, loss of digestive and absorptive capacities (malabsorption) , diarrhoea, crept hyperplasia and recovery. Recovery depends on the severity of the lesion, the relative injury done to the villous cells and crept epithelium, and the age of the animal. It is postulated that the viral infection of the villous epithelial cells redirected cell function from absorption to virus production, and thus the digestive fluids and partially ingested milk accumulated in the

intestinal lumen (Mebus *et al*., 1971; Mebus, 1976 and Woode and Crouch, 1978).

Pohlenz *et al*., (1978) reported that the pathogenesis of cryptosporidiosis is by pushing away the brush border of small intestine (lower part) and colon, which in turn decrease the intestinal enzyme activity. Because the organisms reside within the brush border of intestinal absorptive cells, the primary mechanism for diarrhoea appear to be malabsorption (Moore, 1989). Cryptosporidium oocysts exist within the lumen of the intestine leading to auto or self infection with the sporozoites. This ability of cryptosporidium allows individual animals to be infected persistently. Pohlenz *et al*., (1978) stated that the infection persisted for at least 10 months in one calf infected with cryptosporidiosis.

1. 5. 1. 2 Exudative diarrhoea

Acute or chronic inflammation or necrosis of the intestinal mucosa caused by bacteria, viruses, fungi, protozoa, chemical agents and tumors results in both a net increase in fluid production and inflammatory products, including loss of serum proteins and a reduction in absorption of fluids and electrolytes. The classic example is enteric salmonellosis in which there is severe inflammation with the production of fibrinous, hemorrhagic enteritis. Mebus, (1976) stated that large population of bacteria in the intestinal lumen might also delay restoration of the intestinal epithelial or even cause further epithelial injury. Other notable examples include swine dysentery, bovine virus diarrhoea and inorganic arsenic poisoning. There is also evidence that active electrolyte secretion occurs in enterocolitis is due to salmonellosis in several species of animals. In diseases such as swine dysentery, the permeability of the colon may remain normal or even decrease, but the absorption of water and electrolytes is decreased. This suggests that the primary cause of fluid

and electrolyte loss in some diseases of the colon may be due to failure of the affected epithelium to absorb fluids and electrolytes (Radostits *et al.*, 2000).

Stafseth, (1931); Ott, (1937) and Tyzzer, (1937) reported that in coccidiosis also there is severe destruction of intestinal lining epithelium and these may predispose to secondary bacterial infection by opportunistic pathogen.

1. 5. 1. 3 Secretory diarrhoea

A secretory−absorptive imbalance results in a large net increase in fluid secretion with little if any structural change in the mucosal cells. The enterotoxins produced by enterotoxigenic *E.coli* cause an increase in net secretion of fluid and electrolytes from systemic circulation into the lumen of the intestine (Smith and Hall, 1967). The villi, along with their digestive and absorptive capabilities, remain intact. The crypts also remain intact; however, their secretion is increased beyond the absorptive capacity of the intestines, resulting in diarrhoea. The increased secretion is due to an increase in cyclic adenosine monophosphate which in turns stimulated by prostaglandins.

It is diagnostically useful in enterotoxic colibacillosis when the integrity of the mucosal structure is maintained and the secreted fluid is isotonic, electrolyte rich, alkaline and free of exudates. The outcome is varying degrees of dehydration, electrolyte imbalances, acidosis, hyperkalemia and when acidosis is severe, circulatory failure, shock and death (Radostits *et al.*, 1994).Thus the presence of the histopathological changes such as villous atrophy and severe inflammation in the intestinal epithelium of calves and pigs infected with enterotoxigenic *E. coli* suggests that other substances such as endotoxins may be released from other strains of *E. coli* or from other enteropathogens (Hornich *et al.*, 1975).

1. 5. 1. 4 Abnormal intestinal motility

Hyperexcitability, convulsions and the stress of unexpected sudden confinement may result in diarrhoea which may be due to increased peristalsis resulting in intestinal hurry and reduced intestinal absorption due to rapid passage of intestinal fluids in an otherwise normal intestine. This can occur in animals which are being assembled for transportation and during transportation (Radostits *et al.*, 2000).

1. 6 Pathophysiologic changes

1. 6. 1 Dehydration

The net effect of an increase in the total amount of fluid in the intestinal lumen and a reduction in intestinal absorption is a loss of fluids and electrolytes at the expence of body fluids and electrolytes and the normal intestinal juices. The fluid which is lost consists primarily of water, the electrolytes: sodium; chloride; potassium and bicarbonate, and varying quantities of protein. Protein is lost (protein losing enteropathy) in both acute and chronic inflammation leading to hypoprteinemia in some cases. The loss of bicarbonate results in metabolic acidosis which is of major importance in acute diarrhoea. The loss of sodium, chloride and potassium results in serum electrolyte imbalances. In the horse with enteric sallmonellosis, there is severe dehydration and marked hyponatremia. In the calf with neonatal diarrhoea there are varying degrees of dehydration and a moderate loss of all electrolytes. In acute severe diarrhoea, there is severe acidosis and reduced circulating blood volume. This results in uremia, anaerobic oxidation and lactic acidosis which accentuates the metabolic acidosis. Hyperventilation occurs in some animals in an attempt to compensate for the acidosis.

In acute diarrhoea, large quantities of intestinal fluid are lost in the faeces and large quantities are present in the intestinal lumen (intraluminal dehydration), which accounts for the remarkable clinical

dehydration in some affected animals. The fluid moves out of the intravascular compartment first, then out of the extravascular compartment (interstitial spaces), followed lastly from intracellular space. Thus in acute diarrhoea of sudden onset the actual degree of dehydration present initially may be much more severe than is recognizable clinically; as the diarrhoea continues, the degree of clinical dehydration becomes much more evident.

In chronic enteritis, the intestinal wall becomes thickened and mucus secretion is stimulated, the absorption of the intestinal fluids is also decreased but not of the same magnitude as in acute enteritis. In chronic enteritis, also there is a negative nutrient balance because of decreased digestion of nutrients and decreased absorption resulting in body wasting (Mebus, 1976 and Radostits *et al.*, 2000). The animal may continue to drink and maintain almost normal hydration. In some cases of chronic enteritis, depending on the cause, there is continuous loss of protein leading to clinical hypoproteinemia. Intestinal helminthiasis of all species, John's disease of ruminants and chronic diarrhoeas of the horse are examples.

1. 6. 2 Acidosis

The loss of faecal bicarbonate leads to development of acidosis, Tenant *et al.*, (1972); Lewis *et al.*, (1973) and (1975) found that, acidosis also develops as a result of renal dysfunction, increased microbial activity in response to fermentation of undigested milk as well as increased production and decreased utilization of lactic acid in dehydrated calves.

1. 7 Clinical findings

Dysentery, varying degrees of dehydration and acid base imbalance, abdominal pain, septicemia and toxaemia with fever occur commonly with diarrhoea which is the major clinical findings in enteritis or malabsorption, and their degree of severity depends on the causative

agent and its location, the age and species of animal, and the stage of the disease (Radostits *et al*., 2000).

In acute diarrhoea, the faeces are soft or fluid in consistency and may have an unpleasant odor. They may contain blood (dysentery), fibrinous casts and mucus or obvious foreign material such as sand. The colour of the faeces will vary considerably and usually not representative of a particular disease. However, viral infection is characterized by rapid onset of depression, anorexia and few strings of thick saliva hanging from the lips and then there is a sudden onset of profuse watery diarrhoea, faeces are pale yellow mucoid and may contain flecks of blood (Mebus, 1976), while the syndrome in old ages group can be clinically severe and result in temporary physiologic disturbances as a marked decline in the milk production of cows during the first ten days of infection (woode and crouch, 1978; Fleetwood *et al*., 1989; Durham *et al.*, 1989 and Benfield and Saif, 1990).

In chronic diarrhoea, the faeces are usually soft, homogeneous in consistency, contain considerable mucus and usually do not have a grossly abnormal odor. Progressive weight loss and emaciation are common and there are usually no systemic abnormalities. Animals with chronic diarrhoea will often drink and absorb sufficient water to maintain clinical hydration but there may be laboratory evidence of dehydration and electrolyte loss.

In parasitic infection and abomasitis there may be hypoproteinemia and subcutaneous edema. Weight loss, anorexia, depression, weakness and dehydration are also reported in cryptosporidiosis infection (Pohlenz, 1980).

Rectal body temperatures are usually normal, they can be subnormal in severely ill animals in colibacillosis infection (Zeman *et al*., 1989).

1. 8 Clinical Pathology

The approach to the diagnosis of diarrhoea requires a consideration of the epidemiological history and the nature and severity of the clinical findings. With the exception of the acute diarrhoea in newborn farm animals, most of the other common enteritis have reasonably distinct epidemiological and clinical features. However, the accurate and specific etiological diagnosis of infectious diarrhoeic disease is difficult to obtain. Some reasons of this difficulty are the variety of the incriminated agents, the non specific symptoms manifested and the environmental factors (Logan, 1974 and Acres, 1975). Schoenian, (2009) stated that it is not possible to definitively determine the infectious organism by looking at the colour, consistency, or odor of the faeces. A definitive identification requires a sample for microbiological analysis. Thus, Examination of the faeces is to determine the presence of causative bacteria, helminths, protozoa, viruses and chemical agents and to differentiate them depends on laboratory examination and also, to determine the presence or absence of blood.

In outbreaks of diarrhoea, especially in neonates, it may be useful to do necropsies on selected early untreated cases of acute diarrhoea. Faecal samples should be taken from both normal and affected animals because the comparison of results will improve the accuracy of interpretation.

If possible, a heamogram should be obtained to assist in determining the presence or absence of infection.

1. 9 Treatment

Principles of treatment of enteritis and the consequent diarrhoea are:

1. 9. 1 Removal of the causative agent

Specific treatment is usually directed at intestinal helminthiasis with anthelmintics, antiprotozoan agents against disease like coccidiosis

and antimicrobials agents against the bacterial infections. There are no specific treatments available for the viral diarrhoea in farm animals (Radostits *et al.*, 2000). However, in the absence of complication, recovery from viral infection will occur without specific treatment (Blood *et al.*, 1983). Also for cryptosporidiosis, no effective treatment is known however, supportive therapy containing energy sources is the best means of minimizing starvation and mortality (Moore, 1989).

1. 9. 1. 1 Antimicrobials

The use of antimicrobials either orally or parenterally, or by both routes simultaneously for the treatment of the possible presence or occurrence of enteric or systemic bacterial infections is a controversial subject in both human and veterinary medicine. Parenteral preparations are indicated in animals with acute diarrhoea, toxemia and fever to avoid death. Many antimicrobials when given parenterally, are excreted by the liver into the lumen of the intestine and oral preparations may not be necessary. In cases of sub-acute diarrhoea with minimal systemic effects, the use of an oral preparation may be sufficient. However, oral perpetrations should not be used more than three days to avoid suprainfection and because their use allows the development of multiple drug resistance, which is a major public health concern (Radostits *et al.*, 2000).

Studies of Baljer, (1986) showed that some bacterial strains are resistant to most antibiotics, so that antibiotic therapy is unlikely to succeed unless the sensitivity to the bacteria was tested before treatment. Jones, (1996) also reported that the wide spread and erratic use of broad spectrum antibiotics without proper isolation of the causative agent and drug sensitivity test is a real cause of resistance. McDonnell and Russel, (1999) and Russel, (1999) concluded that, different types of microbes vary in their response to antibiotics and other antimicrobial agents.

However, uses of antibiotics in selected cases of diarrhoea will decrease the symptoms or reduce faecal shedding of the organism and prevent spread of infection. While wrong choice of antimicrobial agents will worsen the symptoms. It may increase the length of time over which affected animals excrete the organisms which may occur in enteric salmonellosis. Thus, risk and benefits should be considered before prescribing antimicrobial agents (Lolekha, 1986).

However, time does not usually permit pretreatment culture of the organism and determination of the drug sensitivity, so that the broad spectrum antibiotics and selected chemotherapeutics based on previously successful experience are used (Blood *et al.*, 1983).

Mass medication of the drinking water supply with antimicrobials for the treatment of outbreaks of specific infectious enteritis in animals is used commonly and with success. One of the best examples is pigs affected with swine dysentery. However, not all animals will drink sufficient quantity of medicated water and daily intake must be monitored carefully. Severely affected animals in an outbreak need individual treatment (Radostits *et al.*, 2000).

1. 9. 2 Alteration of the diet

If the cause of the diarrhoea is dietary in origin, the feed should be removed until the animal has fully recovered; feed should then be replaced by another source or reintroduced gradually when recovery is apparent. The rationale is that, in acute enteritis the digestibility of nutrients is reduced considerably and undigested feed provides a substrate for fermentation and putrefaction to occur, the products of it accentuate the malabsorptive state. Blood *et al.* , (1983) also stated that, it would appear logical not to feed the calf with nutrients such as milk which must be digested but rather to provide readily absorbable substances such as oral glucose and electrolyte mixture.

However, temporary withdrawal of feed presents practical problems especially in the youngs. The temporary removal from the sow of new born piglets affected with acute enteritis presents practical problems and is of doubtful value; similarly with beef calves nursing cows on pasture. With foals it is relatively easy to muzzle them for 24 hours. With weaned piglets affected with weanling diarrhoea and feeder pigs with swine dysentery, it is common practice to reduce the normal daily intake by half for a few days until recovery is apparent, while mature horses affected with diarrhoea should not have access to any feed for at least 24 hours (Radostits *et al.* , 2000), and even in diarrhoeic calves which are being hand fed milk replacer or whole milk it is common beneficial practice to starve for 24—48 hours (Blood *et al.* , 1983).

During the period of temporary starvation, the oral intake of fluids containing glucose and electrolytes is desirable and necessary to assist in maintaining hydration. In newborn calves with diarrhoea, if oral fluid intake is maintained, the total loss of water from faeces and through the kidney is not significantly greater than in normal calves because in diarrhoeic calves the kidney will effectively compensate for faecal losses.

1. 9. 3 Fluids and electrolytes

The initial goals of fluid and electrolyte therapy for the effects of diarrhoea are: the restoration of the body fluids to normal volume, effective osmolality, composition and acid−base balance. The quality and quantity of fluids required to achieve these goals depend on the characteristics of the dehydration and acid−base electrolyte imbalance. Fluids should be given orally whenever possible to save time and expense and to avoid the complications which can arise from longterm parenteral fluid therapy. Also, fluids should be given as early as possible to minimize the degree of dehydration.

The three major abnormalities of dehydration, acidosis and electrolyte deficit are usually corrected simultaneously with fluid therapy. When severe acidosis is suspected, this could be corrected immediately with a hypertonic (5%) solution of bicarbonate given I/V followed by the administration of electrolyte solution in quantities necessary to correct the dehydration because in severe dehydration, equivalent to 10% of BW was losted, hence large amounts of fluids are necessary.

The initial hydration therapy should be given over the first 4−6 hours by continuous intravenous infusion, followed by maintenance therapy for the next 20−24 hours, or for the duration of the diarrhoea if severe. Radostits *et al*., (2000) reported that Horses with acute enteritis have severe hyponatremia, and following fluid therapy may become severely hypokalemic, as evidenced by weakness and muscular tremors. The hypertonic solution of sodium bicarbonate will assist in correcting the hyponatremia but potassium chloride may need to be added to large quantity of fluids given for dehydration; 1 g of potassium chloride added to each liter of fluid will provide an additional 14 mosmol/L (14 mmol/L) of potassium. In preruminant calves with diarrhoea, the total daily requirement is divided into equal doses given every two to four hours (Zeman *et al*. ,1989).

In the early stage of acute diarrhoea and for animals which are not severely dehydrated, the oral route can also be used successfully to correct dehydration and prevent it from becoming worse. Piglets and lambs affected with dehydration are most effectively using balanced electrolyte solutions given S/C at a dose rates of 20 mL/kg BW every 4 hours and orally at 20 mL/kg BW every 2 hours. Mebus, (1976) reported that a simple effective solution for parenteral use is an equal mixture of isotonic saline (0.85%), isotonic dextrose (5%) and isotonic sodium bicarbonate (1.3%).

1. 9. 4 Intestinal protectants and absorbent

According to Radostits *et al*., (2000), Kaolin and pectin mixture are used widely to coat the intestinal mucosa, inhibit secretions and increase the bulk of the faeces in animals with enteritis, while Aiello and Mays, (1998) stated that although kaolin− pectin is claimed to act as demulcent and adsorbent in the treatment of diarrhoea, clinical studies have not demonstrated any benefits from its administration.

1. 9. 5 Anti-diarrhoeal drugs

Anti-diarrhoeal drugs are medicines that relieve and help to control diarrhoea and some of the symptoms that go along with it.

1. 9. 5. 1 Antimotility drugs

Anticholinergic drugs and opiates are available to decrease intestinal motility. The anticholinergic drugs block the action of acetylcholine on smooth muscle and glands. This results in decreased gastric secretion and emptying and a reduction on both segmental and propulsive movements of the intestines. The opiates function by producing an increase in segmentation while reducing propulsive movements in the intestine. The net effect is an increase in resistance to passage of intestinal contents and more complete absorption of both water and nutrients occurs with a subsequent decrease in the frequency of defecation (Radostits *et al.* , 2000). He also stated that there are no reports of clinical trials using antimotality drugs for the treatment of diarrhoea.

1. 9. 5. 2 Antisecretory drugs

Antisecretory drugs are also available for the treatment of diarrhoea due to the hypersecretory activity of enterotoxin produced by bacteria such as enterotoxigenic *E. coli*.

Loperamide hydrochloride given orally to calves with experimentally induced diarrhoea can delay the onset of diarrhoea by its

inhibition of fluid secretion (Radostits *et al.* , 2000). Blood *et al.* , (1983) reported that antiparasympathomimetics also include chlorpromazine which is antagonistic to enterotoxin cell system and acetylsalsalic acid which reduces fluid accumulation while other antisecretory drugs include, opiates, atropine, prostaglandin inhibitors have not been yet adequately evaluated and the provision of balanced fluids and electrolytes, containing sodium chloride, sodium bicarbonate, potassium chloride and glucose, given both parentally and orally, are considered to be adequate and effective for treating the effects of the hypersecretion (Radostits *et al.* , 2000).

1.10 Control

The control and prevention of enteritis in farm animals is a major topic and activity of large animal practice. Because of the complex nature of disease, it is unrealistic to expect total prevention and control at economical level should be a major goal.

However, Radostits *et al.* , (2000) concluded to that the principles of control include the following:

- Reduce infection pressure by controlling population density.
- Ensure adequate non−specific resistance by adequate colostrum intake of neonatal farm animals and maintaining adequate nutritional status. Worth to notice that protection against enteropathogens depends upon the antibodies within the lumen of the digestive system thus the colostrum must contain reasonably higher level of antibodies and it must be fed every day during crucial age period of susceptibility to be effective (Blood *et al*. , 1983 and Sanders, 1985). McBeath, (1971) found that multiple small intakes of colostrums give significantly a greater level of immunoglobulins than single large intake. Saif and Smith, (1983) suggested that subclinical infection of neonates with rotavirus may occur at birth or shortly thereafter as long as adequate levels of colostral, milk

or serum antibodies persist. Colostrum provides protection against colisepticaemia in calves but does not prevent diarrhoea (Logan, 1973). However, feeding immune colostrum can delay onset of diarrhoea, reduce its incidence, duration and severity and consequently improve live weight (Snodgrass *et al.* , 1982). Evidences available which suggest that rotavirus antibody in milk can protect against small challenge dose and maternal immunization against rotavirus may be a practical proposition (Snodgrass *et al.* , 1980). Saif and Smith, (1983) also, stated that daily feeding (two times/ day) of as little as 40 ml (total) of high tittered colostrum to calves completely protected them against diarrhoea and rotavirus shedding when challenged.

Bachmann, (1982) reported that high serum antibody levels can provide some protection through the leakage back into the intestine while Snodgrass and Wells (1978a) studied the effect of serum on the course of rotaviral infections to lambs; they found that serum or serum products of high antibody titers against rotavirus could replace colostrum if it's not available. The duration of the immunity is not known (Blood *et al.* ,1983). Thus as immunity may be only partial and not long lasting, adults may be diseased from infected neonates, two cows with severe diarrhoea whose calves had calf scours, were shown to be infected with rotavirus indicating that there is no age resistance for rotavirus enteritis in cattle breed (Woode and Bridger, 1975).

Serum antibodies offer a comparable model for antibodies in colostrum, it is also possible that serum or serum products may themselves be a feasible method for rotaviral prophylaxis or therapy. Hyper-immune serum intraperitoneally or orally may be providing a method for protecting neonatal calves against rotaviral diarrhoea (100% protection) (Wells *et al.* , 1978).

- Vaccinate for those diseases for which there is an effective vaccine. The ultimate impact of vaccines on animal's production will depend on their cost effectiveness as compared to the alternate means of control. Active or passive immunizations are both used as vaccine programmes against neonatal calf diarrhoea.

Parental vaccination of the pregnant dam before parturition may increase the level and the duration of colostral vaccine immunity (Snodgrass and Wells, 1978b; Snodgrass, 1984). Baljer (1986) and Castrucci *et al.* , (1987) in a field trial reported that morbidity and mortality rates of neonatal diarrhoea of calves, after introduction of maternal immunization, had been significantly decreased in those received colostrums from vaccinated dams. Trials have also been made to actively immunization either by parental injection of vaccine or orally. Oral vaccines for calves against rotavirus and coronavirus are available but their efficiency has been debated (Deleeuw, 1980; Walter−Toews *et al.*, 1985) , by contrast good results had been obtained from active oral immunization of newborn calves with heat inactivated enterotoxigenic *E. coli* (Baljer, 1986). Rotavirus neutralizing antibody was associated with IgG in both colostrum and milk suggesting that vaccination of the dam may be of value in protecting the suckled lamb against rotavirus infection (Wells *et al.*, 1978). Snodgrass and Wells (1978a) studied the effect of serum on the course of rotaviral infections to lambs; he found that serum or serum products of high antibody titers against rotavirus could replace colostrum if it's not available.

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method for protecting neonatal calves against rotaviral diarrhoea (100% protection) (Wells *et al.* , 1978).

The prognosis of *E. coli* infections is unfavorable if the level of immunoglobulins is low regardless of intensive fluid and antimicrobial therapy (Blood *et al*. ,1983). Vaccination is coming into routine use as part of programmes to control ETEC infections in farm animals in U.S.A. Most of the field trials that have been reported, have presented evidence that vaccination can reduce morbidity and mortality due to naturally occurring ETEC infection. Under some regimes, protection of suckling can be enhanced if dams are vaccinated orally than parentaraly (Collin, 1974).

Nonliving vaccine contain components to stimulate both anti bacterial and antitoxic immunity were added to animal feed. Multiple oral doses from nonliving vaccine are required to prime the secretory immune system and assure a good local antibody response.

Vaccination in-utero was used to induce active immunity in calves, but this method is totally impractical under field conditions (Newman *et al.* , 1978).

- Minimize managemental and environmental stressors. Mebus, (1976) stated that introduction of new animals of any age shortly before or during calving should be discouraged. Janke, (1989) found that identification and removal of persistently infected carrier animals is a priority in control of viral enteritis.

Suckling calves survived better than fed calves because they do not acquire the same high levels of serum immunoglobulins, however, in both cases the presence of the dam improves the absorption (Umoh, 1982 and Blood, *et al.*, 1983).

- Monitor morbidity and mortality and ensure that a diagnosis is obtained so that control measures for newly introduced diseases into a herd can be instituted.

1. 11 Plants with anti-diarrhoeal effects

Atta and Mouneir, (2004) found that oral administration of methanolic extracts of six Egyptian medicinal plants from *Conyza dioscoridis* (CD), *Alhagi maurorum* (AM), *Mentha microphylla* (MM), *Zygophyllum album* (ZA), and *Conyza linifolia* (CL) exhibited a significant (P<0.01) anti-diarrhoeal effect against castor oil-induced diarrhoea at different doses, 200 and 400mg/kg while *Convolvulus arvensis* (CA) produced no anti-diarrhoeal effect in rats at all doses.

The anti-diarrhoeal activity of *Hypoxis hemerocallidea* corm aqueous extract (APE) which is known as ("African potato") was investigated on experimentally-induced diarrhoea in rodents at doses, 50 and 400 mg/kg, given orally and produced a dose-dependent and significant (p < 0.05-0.01) protection of rats and mice against castor oil-induced diarrhoea, inhibited intestinal transit and delayed gastric emptying. This finding supports the use of 'African potato' as a natural supplementary remedy for the treatment, management and/or control of diarrhoea in some rural communities of southern Africa (Ojewole *et al*., 2008).

Agbor *et al.,* (2003) reported that *Alchornea cordifolia* which is quoted by many traditional healers in Cameroon as a plant with anti-diarrhoeal activity, its leaf extract was investigated against castor oil induced diarrhoea in mice at doses 100, 200, 400 and 800 mg/kg, using morphine as the standard reference drug. A significant (p < 0.01) dose inhibited the production of diarrhoeal faeces, was observed with 800 mg/kg, being the most effective. Phytochemical screening revealed the presence of tannins and flavonoids which may account for the increased

colonic water and electrolyte reabsorption, a mechanism suggested for the anti-diarrhoeal activity of *A. cordifolia*.

Ethnobotanical studies indicate that *Helianthemum glomeratum* is a plant widely used in Maya communities to treat diarrhoeas (Meckes *et al*., 1996).

Anti-diarrhoeal activity of *Sclerocarya birrea* bark extract and its active tannin constituent in rats was investigated by Galvez *et al*., (1991), the lyophilized decoction demonstrated anti-diarrhoeal activity in experimental models of diarrhoea induced by magnesium sulphate and sodium picosulphate. Condensed tannin was isolated from the crude drug which produced inhibition in intestinal motility, and the monomer of which was identified as procyanidin.

Shah *et al*., (2009), reported that in an *in vivo* study the crude extract of *Alstonia scholaris* (As.Cr), which tested positive for the presence of alkaloids, provided 31-84% protection against castor oil-induced diarrhoea in mice at 100 and 1000 mg/kg doses, similar to loperamide.

Studies of Akah *et al*., (1998) have demonstrated a potential anti-diarrhoeal activity of the aqueous (WE) and ethanol (EE) leaf extracts of *Pentaclethra macrophylla* using experimental animal models. Anti-diarrhoeal of the extracts was evidenced by a significant reduction in faecal output and protection from castor oil-induced diarrhoea in rats treated with the extracts. The antispasmodic as well as the antimicrobial effects of the extracts possessed, may explain the rationale for the use of the plant in traditional medicine as a popular anti-diarrhoeal recipe.

Murugesan *et al*.**,** **(**1999) evaluated the anti-diarrhoeal profile of a methanol extract of the aerial parts of *Jussiaea suffruticosa* Linn. with several experimental models of diarrhoea in rats. The extract exhibited significant anti-diarrhoeal potential at doses of 100, 200 and 300 mg/kg in

all the animal models and thus established the efficacy of MEJS as a potent anti-diarrhoeal agent.

Seven plants extracts namely: the aerial parts of *Euphorbia paralias* L. (EP), *Bidens bipinnata* L. (BB), *Cynachum acutum* L. (CyAc), *Diplotaxis acris* (Forssk.) Boiss (DA), *Convolvulus fatmensis* (CF) and *Schouwia thebaica* Webb (ST) and the leaves of *Plantago major* L. (PM) were evaluated for their anti-diarrhoeal effects on castor oil-induced diarrhoea. A significant anti-diarrhoeal effect of the tested plant extracts against castor oil-induced diarrhoea in rats was achieved by 200 and 400 mg/kg. Tannins, flavonoids, unsaturated sterols/triterpenes, carboy-rates, lactones and proteins/amino acids were reported as major active constituents of the tested plants (Atta and Mouneir, 2004).

Studies were carried out on castor oil-induced diarrhoea in mice to evaluate the anti-diarrhoeal effect of the aqueous root extract of *Terminalia avicennoides* in rodents. The results revealed that the extract (100, 200 and 400 mg/kg) markedly protected mice against castor oil-induced diarrhoea. A preliminary phytochemical screening of the aqueous extract of *T. avicennoides* roots revealed the presence of tannins, saponins and flavonoids. The results obtained showed that the water extract of *T. avicennoides* roots may contain some biologically active principles that may be active against diarrhoea and this may be the basis for its use traditionally for gastrointestinal disorders (Abdullahi *et al.*, 2001).

Besra *et al.*, (2000) found that the aqueous methanol extract of *Albizzia lebbeck* seeds (2.5 and 5 mg/kg i.p.) possessed anti-diarrhoeal activity against castor oil-induced diarrhoea in rodents, which strengthens the earlier use of the seeds in the treatment of diarrhoea and dysentery.

The anti-diarrhoeal activity of a procyanidin isolated from the bark of *Sclerocarya birrea* was studied, using four models of experimentally induced diarrhoea in rats. The cathartic agents used were: magnesium

sulphate, castor oil, arachidonic acid and prostaglandin E_2. At doses of 150 mg/kg, the procyanidin showed anti-diarrhoeal activity in all the models of experimentally induced diarrhoea (Galvez *et al.,* 1992).

The bark of *Myracrodruon urundeuva* (family Anacardiaceae), which is used in popular medicine as an anti-diarrhoeal agent, has been investigated for its effects on castor oil-induced diarrhoea. In rats, the ethanol extract of stem bark significantly inhibited the castor oil-induced diarrhoea, at an oral dose of 400 mg/kg (Chaves *et al.,* 1998).

Kumar *et al.,* (2001) reported that indigenous Indian plants such as *Andrographalis paniculata*, *Asparagus racemosus*, *Butea monosperma*, *Cassia auriculata*, *Ficus hispida*, *Hemidesmus indicus* and *Guiera senegalensi are* widely used to treat diarrhoea.

1. 12 Plant used in the present study

1. 12. 1 *Hydnora abyssinica* A. Braun ex Schweinf. : Figure (1)

Hydnora abyssinica A. Br. (family Hydnoraceace) is the accepted name in African Plant Checklist and Database.

Beentje and Luke, (2002) reported that there were 13 synonymy:

- *Hydnora johannis* Becc. [family Hydnoraceae]
- *Hydnora cornii* Vacc. [family Hydnoraceae]
- *Hydnora michaelis* Peter [family Hydnoraceae]
- *Hydnora abyssinica* A.Braun var. quinquefida Engl. [family Hydnoraceae]
- *Hydnora bogosensis* Becc. [family Hydnoraceae]
- *Hydnora aethiopica* Decne. [family Hydnoraceae]
- *Hydnora johannis* Becc. forma forma trimera Vacc. [family Hydnoraceae]
- *Hydnora ruspolii* Chiov. [family Hydnoraceae]
- *Hydnora johannis* forma forma gigantea Becc. [family Hydnoraceae]

(Un- opened flower)

(Un- opened flower broken by animal) Tarangire, Tanzania

Fig. (1): Hydnora abyssinica

These photos belongs to kibuyu's photo stream (2,155) .

December 2009 in Babati, Manyara, TZ

http://www.flickr.com/photos

Fig. (2): Hydnora abyssinica A. Braun ex Schweinf

Opened flower

Photo: Cathy Sharp

Nr Mvuma

www.zimbabweflora.co.zw/speciesdata/species.

Fig.(3): Hydnora abyssinica Schweinf.

275 × 585 - 7k – gif

www. puka.cs.waikato.ac.nz

- *Hydnora hanningtonii* Rendle [family Hydnoraceae]
- *Hydnora gigantea* Chiov. [family Hydnoraceae]
- *Hydnora johannis* Becc. var. quinquefida (Engl.) Solms [family Hydnoraceae]
- *Hydnora solmsiana* Dinter [family Hydnoraceae]

1. 12. 2 Derivaion of specific name

Abyssinica of Abyssinia (Ethiopia) (Hyde and Wursten, 2010).

1.12. 3 Repository

Royal Botanic Gardens, Kew (K) (Beentje and Luke, 2002).

1. 12. 4 Collections

Flora of Tropical East Africa (Beentje and Luke, 2002).

1. 12. 5 Distribution and Vernacular name

Kenya, Tanzania, Uganda, Botswana, Zimbabwe, Ethiopia, Somalia, South Africa, Sudan, Swaziland, Tanzania, Uganda, Yemen, the Democratic Republic of Congo, Namibia, Namibia, Rwanda and Eritrea (Beentje and Luke, 2002).

Distribution in Sudan: Central Sudan and locally known as Tartous.

1. 12. 6 Habitat

In Acacia woodland and scrub, Acacia-Commiphora scrub and grassland with scattered Acacia spp., usually on black cotton, clay and sandy alluvial soils, also on rocky slopes. Parasitic on Acacia spp. but also reported on Albizia, Delonix and probably Commiphora spp. (Musselman, 1997).

1. 12. 7 Description

A Subterranean root parasite, lacking chlorophyll. Plant body (‘branch’) rhizome-like, simple or with widely spreading horizontal branches, fleshy, irregularly shaped, somewhat flattened, brown outside,

brick-red or reddish pink to white inside, with sticky, astringent exudates when fresh, rich in tannins. Flowers emerging directly from the main and side branches, remaining partially below ground and partially emerging, solitary or in groups, but only one per branch emergence point , with very foetid smell; pedicel absent. Perianth fleshy-coriaceous, Fruit entirely subterranean, fleshy, globose, many-seeded, often splitting irregularly at maturity; outer periderm scaly; inner pericarp mealy, white; seeds brown, oblong to globos. Seedlings are unknown. Plants usually flower after the onset of the rains. Flowers are extremely fetid, visited by beetles. Fruits develop about five months after the flowers appear, are scaly brown and entirely subterranean. The species mostly parasitizes Acacia spp. Plants may be more common than recorded, as they are only visible when flowering (Musselman, 1997).

1. 12. 8 Traditional uses

Fruits are sweet tasting and eaten by animals and people, with white flesh and abundant brown seeds. Other parts of the plant are eaten by animals (Hyde and Wursten, 2010).

1. 12. 8 Medicinal uses

Roots are used as medicine for diarrhoea and to staunch hemorrhaging (Hyde and Wursten, 2010).

CHAPTER TWO
MATERIALS AND METHODS

2. 1 Materials and experimental designs

Two experiments were performed in the testing plant material using rats.

2. 1. 1 Anti-diarrhoeal activity of *Hydnora abyssinica* aqueous extract on rats

2. 1. 1. 1 Plant material

The collected *Hydnora abyssinica* roots were identified and authenticated in the Department of Botany, Faculty of Science, University of Khartoum.

2. 1. 1. 2 Animals, housing and management

Twenty four Wister white (Albino) rats of either sex weighing 100−150gm were obtained from the Animals House Unit of the Medicinal and Aromatic Plants Research Institute (MAPRI), National Center for Research Khartoum, Sudan, where they were housed in cages (each of dimensions 12×12×12cm. accommodating one dose–group) and maintained in a room under standard environmental conditions (controlled temperature (22±2ºC) and relative humidity (60%)) with free access to water and formula rat feed (2.5 Mcal and 20% crude protein).

Animals were apparently healthy and they were identified by colour tail marks. One week was allowed as a preliminary adaptive period.

2. 1. 1. 3 Administration and doses

At the end of the adaptation period, rats were divided randomly to five groups each of five rats. They were fasted for 18 hours with free access to water prior to the commencement of the experiment. **All**

groups' individuals were given 1 ml/kg body wt. /rat caster oil orally using orogastric canulla as an acute diarrhoeal inducer 30 minutes subsequent to extract administration. **Group1** rats were the un-treated control and received orally 1ml/kg body wt. /rat normal saline. *Hydnora abyssinica* roots aqueous extract was redissolved in distilled water and given orally to rats of **Group 2** at 100 mg/kg body wt., 200 mg/kg body wt. to rats of **Group 3** and 400 mg/kg body wt. to rats of **Group 4**, while rats in **Group 5** were treated with Loperamide (Hikma Pharmaceutical, Amman, Jordan), 3 mg/kg body wt. /rat, p.o, as a reference anti-diarrhoeal compound. Each rat was then housed separately in a cage over clean filter paper. Then diarrhoea episodes were observed for a period of 24 hours.

2. 1. 1. 4 Parameters

Clinical signs and mortality rates were recorded. First defecation time and frequency of defecation were also recorded. Total weight of faecal mass was collected successively and weighed at 4 and 6 hours post administration of castor oil. Anti-diarrhoeal activity was determined in terms of percentage reduction in cumulative faecal mass with respect to un-treated control group (Patel *et al*., 2006).

Blood samples were obtained from the ocular vein after the start of experimental dosing and thereafter 24 hours at slaughter with and without anticoagulant for haematological investigations and serum analysis respectively. Haemoglobin concentration (Hb), packed cell volume (PCV) and red blood cells (RBCs) counts were estimated. Mean corpuscular volume (MCV) and mean corpuscular Haemoglobin concentration (MCHC) were calculated.

Sera were analyzed for the concentration of total protein, albumin, sodium, potassium and calcium.

2. 1. 2 Toxicity of *Hydnora abyssinica* aqueous extract to Albino rats

2. 1. 2. 1 Plant material

Hydnora abyssinica roots after collection were identified and authenticated in the Department of Botany, Faculty of Science, University of Khartoum.

2. 1. 2. 2 Animals, housing and management

Twenty Wister white (Albino) rats of either sex weighing 100-130 gm were obtained from the Animals House Unit of the Medicinal and Aromatic Plants Research Institute, National Center for Research Khartoum, Sudan, where they were housed in cages (each of dimensions 12×12×12cm. accommodating one dose-group) and maintained in a room under standard environmental conditions (controlled temperature (22±2ºC) and relative humidity (60%)) with water provided *ad libitum* and formula rat feed (2.5 Mcal and 20% crude protein). Animals were apparently healthy and they were identified by colour tail marks. Experimental animals were allowed one week as a preliminary adaptive period.

2. 1. 2. 3 Administration and dose rates

Post adaptive period, the animals were allotted randomly to four groups, each of five rats. Rats in **Group 1** were the un-dosed control. *Hydnora abyssinica* aqueous extract was redissolved in distilled water and given orally (p.o) in daily doses at 400 mg/kg body wt. /rat to **Group 2**. Rats in **Group 3** received 800 mg/kg body wt./rat/day, while rats in **Group 4** received 1600 mg/kg body wt./rat/day. Dosing continued for seven days.

2. 1. 2. 4 Parameters

Clinical signs and state of living were recorded. The animals were observed for deaths and manifestation of toxic effects which include: agility, muscular tremors and convulsions. Breathing patterns, water and

food intake are also noticed. Blood samples were obtained from the orbital plexus with and without anticoagulant before the start of the experimental dosing and at slaughter for haematological examination and serum analysis. Haemoglobin concentration (Hb), packed cell volume (PCV) and red blood cells (RBCs) counts were measured. Mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were also calculated.

Sera were analyzed for the activities of (AST), (ALT) and also for the concentration of metabolic indicators such as total protein, albumin, sodium and potassium. Rats were anaesthetized with diethyl ether and sacrified at the end of the week. Tissues were examined immediately to identify lesions.

2. 2 Methods

2. 2. 1 Preparation of the plant extract

Hydnora abyssinica roots were cleaned; shade dried and pulverized using pestle and mortar. A total of 500g coarse powder of the plant material obtained was successively extracted at the Medicinal and Aromatic Plants Institute (MAPRI), by soaking into boiling distilled water and left for 4 hours at room temperature, the mixture was occasionally shaken then filtered and the filtrates were cooled over night at −4ºC and concentrated. The concentrated extract was then freeze dried (Komolafe *et al.,* 1988). The extract obtained was glittery powder, brown greenish in colour and weighed 70gm, the yield percentage was calculated. Then the extract obtained was stored in a closed container till use.

2. 2. 2 Haematological methods

These were described by Schalm (1965). Blood samples from rats were collected into clean dry bottles containing the anti-coagulant heparin from the ocular veins.

2. 2. 2. 1 Haemoglobin (Hb) concentration

The concentration of haemoglobin was determined by the methaemoglobin technique. The method is based on the conversion of haemoglobin by Drabkins solution (0.2g of potassium cyanide, 0.2g of potassium ferricyanide and 1g of sodium bicarbonate per liter of distilled water) to cyanomethaemoglobin. The haemoglobin concentration was estimated in g/dl of blood.

2. 2. 2. 2 Packed Cell Volume (PCV)

Fresh blood samples were drawn in capillary tubes and centrifuged in a microhaematocrit centrifuge (Hawksley and Sons Ltd. England) for 5 minutes. Packed Cell Volume percent was read off on the scaling instrument provided with the centrifuge.

2. 2. 2. 3 Red Blood Cells (RBCs) counts

Red blood cells were counted using an improved Neubauer haemocytometer (Hawksley and Sons Ltd. England). Formal citrate was used as a diluent.

2. 2. 2. 4 Mean Corpuscular Volume (MCV)

The MCV was calculated from the PCV and RBCs values as follows:

$$MCV(fl) = \frac{PCV\%}{RBC_s(million/ml)} \times 10$$

2. 2. 2. 5 Mean Corpuscular Haemoglobin Concentration (MCHC)

MCHC was calculated from Hb and PCV values as follows:

$$MCHC(\%) = \frac{Hb(g/dl)}{PCV(fl)} \times 100$$

2. 2. 3 Chemical methods

Blood samples obtained, initially and a week thereafter, from the ocular vein of rats on dosing with the test extract were used to prepare sera for the chemical methods. Venous blood samples were allowed to clot. Serum was separated by centrifugation at 3000 r.p.m. for 5 minutes and stored at -20ºC until analyzed. Spectrophotometer, Merck Mega, Version 0.6, 1995 (E. Merck, Darmstadt, Germany) was used to analyze serum activities of enzymes AST and ALT and serum metabolites; total protein, albumin, sodium, potassium and calcium.

2. 2. 3. 1 Total protein

Total serum protein concentration was measured by a colourimetric method using a commercial kit (Randox Laboratories Ltd., U.K.).

Test principle

Colourimetric determination of total protein in serum is based on the Biuret reaction. The serum protein reacts with copper sulphate in the presence of sodium hydroxide. The Rochelle salt (K-Na-tartarate) contained in the Biuret reagent is utilized to keep the formed cupric hydroxide in solution which gives the blue colour. The intensity of the colour produced is proportional to the amount of protein in the sample. The absorbencies of the sample (A sample) and of the standard (A standard) were read against the reagent blank in the Spectrophotometer at a wavelength of 545*nm*. The total serum protein concentration (C) was calculated as follows:

$$C(g/dl) = \frac{A\ sample}{A\ standard} \times concentration\ of\ standard$$

2. 2. 3. 2 Albumin

Serum albumin was measured by a colourimetric method using a commercial kit (Roandox Laboratories Ltd., U.K.).

Test principle

The measurement of serum albumin is based on its quantitative binding to the indicator 5, 5-dibromo- o-cresolsulphonphthalin (bromocresol purple, BCP).

Serum was mixed with a buffered BCP reagent and the mixture was incubated for 2 minutes at room temperature. The absorbance of the sample (A sample) and of the standard (A standard) was measured against the reagent blank at a wavelength of 600*nm* and albumin concentration (C) was calculated as follows:

$$C(g/dl) = \frac{A\ sample}{A\ standard} \times concentration\ of\ standard$$

2. 2. 3. 3 Sodium

Serum Sodium concentration was determined by flame photometry as described by Varley (1967). The method is based on passing, under controlled conditions, a diluted serum (1:100) as a very fine spray in the air supply to a burner where the solution evaporates and the salt dissociates to give neutral atoms. Light of characteristic wavelength was emitted and passed through a specific filter for sodium on to a photocell and the amount of current produced was read on galvanometer. The air pressure was adjusted to 10 *lb* per square inch and butane gas was used for the flame.

The spray was formed by passing compressed air through an atomizer into which the diluted serum was drawn by suction and then entered the burner with its air supply. The gas and air pressures were

carefully regulated in order to maintain a constant and steady flame. Changes in the galvanometer readings were recorded and values were estimated in mEq/Litre as follows:

$$Sodium\ concentration\ (mEq/L) = \frac{T}{S} \times 140$$

The values were then converted to mg/100ml.

T = tested; S = standard and 140 = factor.

2. 2. 3. 4 Potassium

The method described by Varley (1967) for the determination of serum potassium is based on the same principle of flame photometry as in case of serum sodium. The sensitivity of the instrument was varied in order to use the same dilution of serum (1:100) for both sodium and potassium. Light of characteristic wavelengths was emitted and passed through a specific filter for potassium on to a selenium cell and the amount of current produced was measured. Changes in the galvanometer readings were recorded and values were estimated in mEq/liter as follows:

$$Potassium\ concentration(mEq/L) = \frac{T}{S} \times 5$$

The values were then converted to mg/100ml.

T = tested; S = standard and 5 = factor.

2. 2. 3. 5 Calcium

Serum calcium concentration was determined by a colourimetric method using a commercial kit (Randox Laboratories Ltd., U.K.).

Test principle

Calcium ions form a violet complex with chromogen (O-Cretholphthalein complexone -8- hydroxyquinoline hydrochloric acid) in an alkaline medium (2- amino- 2- methyl- propan- 1- 01).

Serum was mixed with a buffered reagent and the absorbance of the sample (A sample) and of the standard (A standard) was measured against reagent blank at wavelength of 578*nm* and calcium concentration (C) was calculated as follows:

$$C(g/dl) = \frac{A\ sample}{A\ standard} \times concentration\ of\ standard$$

2. 2. 3. 6 Glutamate oxaloacetate transaminase (Aspartate aminotransferase, L-Aspartate; 2- oxoglutarate aminotransferase, E. C. 6. 1. 1; GOT, AST)

Serum AST activity was measured by a commercial kit (Randox Laboratories Ltd, U.K.).

Test principle

Aspartate aminotransferase catalyses the reversible transfer of an amino group from aspartate to α-ketoglutarate forming glutamate and oxalacetate:

L-aspartate + α-ketoglutarate ←GOT→ L-glutamate + oxalacetate

The oxalacetate produced is reduced to malate by dehydrogenase MDH and NADH.

Oxalacetate + NADH + H^+ ← NADH → L-Malate + NAD

The rate of decrease in concentration of NADH is proportional to the catalytic concentration of AST present in the serum sample.

Protocol:

Non-haemolysed serum was added to a buffered substrate mixture of L-aspartate and α-oxoglutarate. The absorbance at a wavelength of 365*nm* was read at one minute intervals after mixing the serum with the buffered substrate solution. The mean absorbance change per minute (A_{365}/minute) was used for calculation of enzyme activity as follows:

IU = A_{365} nm/minute × 2059

2. 2. 3. 7 Alanine amino transferase, ALT (Glutamic pyruvic transaminase, L- aspartate, 2- oxoglutamate, GPT)

It is enzymatic method, which measures glutamic pyruvic transaminase in sera by monitoring the concentration of pyruvic hydrazone formed with 2-4 dinitrophenyl hydrazine.

Test principle:

Oxoglutarate + L-alanine ←GPT→ L-glutarate + Pyruvate

The absorbance of samples was read against the reagent blank after 5 minutes at wavelength of the 630*nm* UV/VIS Spectrophotometer. The GPT was measured in U/L.

2. 2. 4 Statistical methods

Data derived from blood, sera and faeces' weight were expressed as means ± S.E. Statistical differences between mean values were analyzed by a completely randomized design; using one way ANOVA (Analyses of Variance) followed by Duncan's Multiple Range Test with aid of SAS computer programme (SAS, 1998). $P<0.05$ was considered significant. The efficacies were obtained by calculating the differences between the faeces weight in the treated and the control group and the values were transformed into percentage using mean index in the formula:

(a-b)/a*100 = efficacy

Were **a** is mean of the faeces weight in the control and **b** the faeces weight in the treated rats (Snedecor and Cochran, 1989).

CHAPTER THREE
RESULTS

3. 1 Effect of *Hydnora abyssinica* roots aqueous extract on castor oil induced diarrhoea in rats

3. 1. 1 Clinical signs

Rats in groups, 2 (100mg/kg), 3 (200mg/kg) and 4 (400mg/kg) showed no clinical sings during the experimental period (24 hours) and no mortalities were recorded. Group 5, which is treated, showed also normal behavior and no mortalities. In the un-treated control rats (group 1) there were copious diarrhoea seen but also no mortalities were recorded.

3. 1. 2 Pathological changes

For all test groups 2, 3, 4 and 5 no gross changes were observed at postmortem at the end of the experiment (24 hours). Normal organs were also observed in the control group (group 1).

3. 1. 3 Effect of *Hydnora abyssinica* roots aqueous extract on faecal weight of diarrhoeic rats

The effects of the aqueous extract of *Hydnora abyssinica* roots on castor oil induced diarrhoea in rats are shown in **Table (1).** The effect of the plant extract on weight of faeces and the inhibition percentage the extract produced are shown in **figure (4)** and **figure (5)** respectively.

The aqueous extract of *Hydnora abyssinica* exhibited marked dose dependent anti-diarrhoeal activity in the study. The extract significantly inhibited both the frequency of defecation as well as the weight of faecal matter in rats.

Rats of group 2(100mg/kg) demonstrated a significant ($P<0.05$) decrease in faeces weight in the fourth and sixth hours, as compared with

the control group (castor oil group), showing efficacy rates of 46.78 and 27.42% respectively.

Group 3(200mg/kg) rats indicated significant (*P<0.05*) difference in weight of faeces when compared with the control group (un-treated group) in the fourth and sixth hours with inhibition percentage of 63.21 and 50.88% respectively.

Rats of group 4(400mg/kg) showed highly significant (*P<0.05–0.001*) decrease in weight of faeces when compared with the control group (un-treated group) at the fourth and sixth hours and providing highly inhibition rates of 74.68 and 60.13% respectively.

Group 5 (Loperamide) rats also showed highly significant (*P<0.05–0.001*) difference in the weight of faeces when compared with the control group (un-treated group) in the fourth and sixth hours and recorded higher inhibition percentage of 66.54 and 72.21% respectively.

Generally, the highest inhibition percentage obtained in this study was 74.68% with the dose 400mg/kg body weight of the extract which was comparatively greater than 72.21%, the maximum inhibition percentage produced by loperamide.

3. 1. 4 Changes in serum metabolites

Table 2 and **Table 3** show the changes in serum metabolites of rats treated orally with *Hydnora abyssinica* roots aqueous extract at 4 and 24 hours respectively.

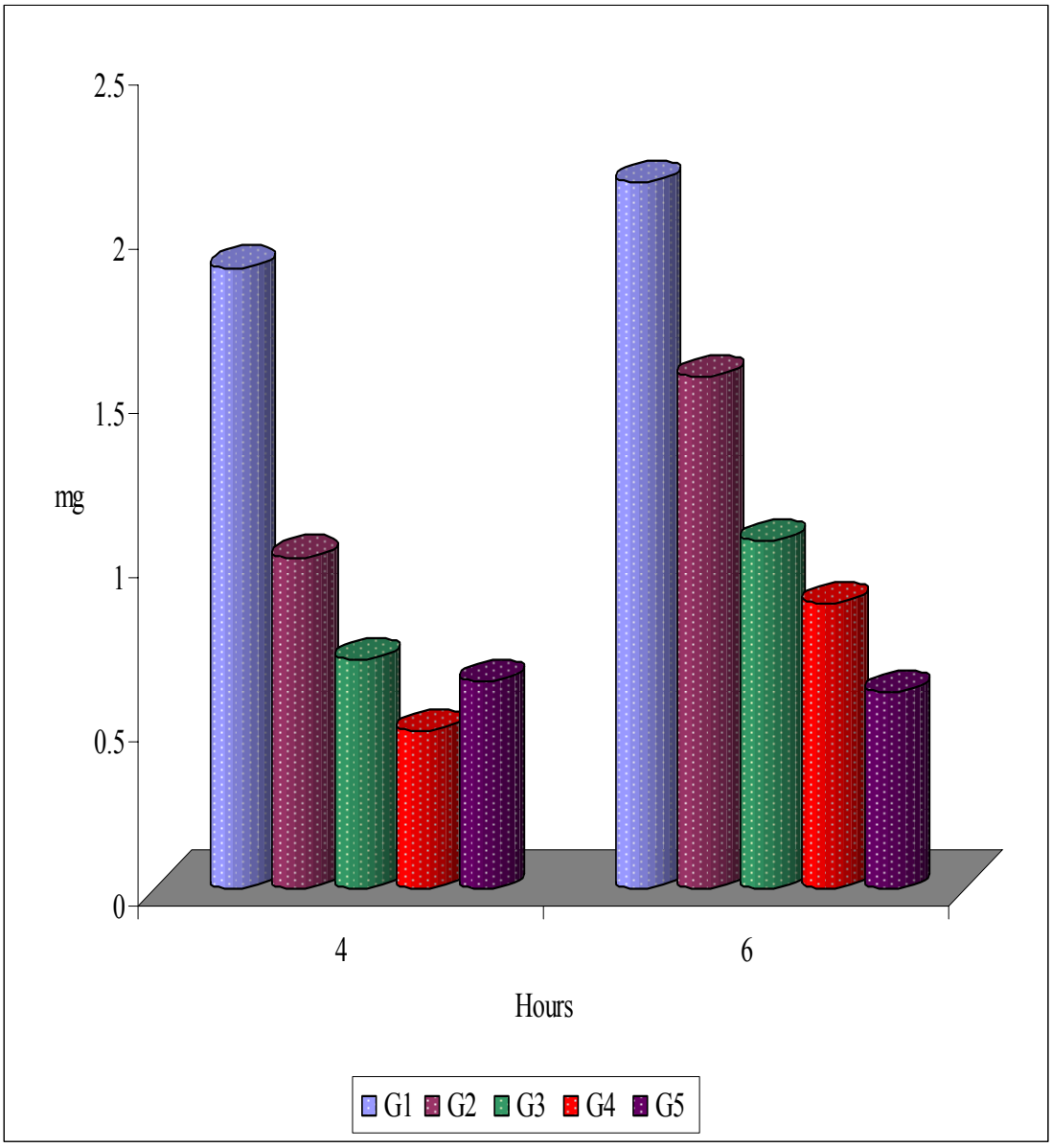
At 4 hours, the values of total protein and sodium showed significant (*P<0.05*) increase in groups 3, 4 and 5 when compared with the control group. Also, the values of albumin in groups 4 and 5 demonstrated significant (*P<0.05*) increase. The concentration of calcium only in groups 4 and 5 showed significant (*P<0.05*) decrease while potassium in all groups demonstrated significant increase when compared with the control group.

Table 1. Effect of *Hydnora abyssinica* roots aqueous extract on castor oil–induced diarrhoea in rats

Hours	Groups	Dose mg/kg	Faeces weight mg	Inhibition %
4hrs	G1	un-treated control (saline)+ castor oil	01.89± 00.01 **a**	**00.00**
	G2	100mg/kg *H.aby.* + castor oil	01.01 ± 00.00 **b**	**46.78**
	G3	200mg/kg *H.aby.* + castor oil	00.70 ± 00.03 **c**	**63.21**
	G4	400mg/kg *H.aby.* + castor oil	00.48 ± 00.01 **e**	**74.68**
	G5	3mg/kgLopramide +castor oil	00.63 ± 00.01 **d**	**66.54**
6hrs	G1	un-treated control (saline)+ castor oil	02.15 ± 00.00 **a**	**00.00**
	G2	100mg/kg *H.aby.* + castor oil	01.56 ± 00.01 **b**	**27.42**
	G3	200mg/kg *H.aby.* + castor oil	01.06 ± 00.02 **c**	**50.88**
	G4	400mg/kg *H.aby.* + castor oil	00.87 ± 00.03 **d**	**60.13**
	G5	3mg/kgLopramide +castor oil	00.60 ± 00.00 **e**	**72.21**

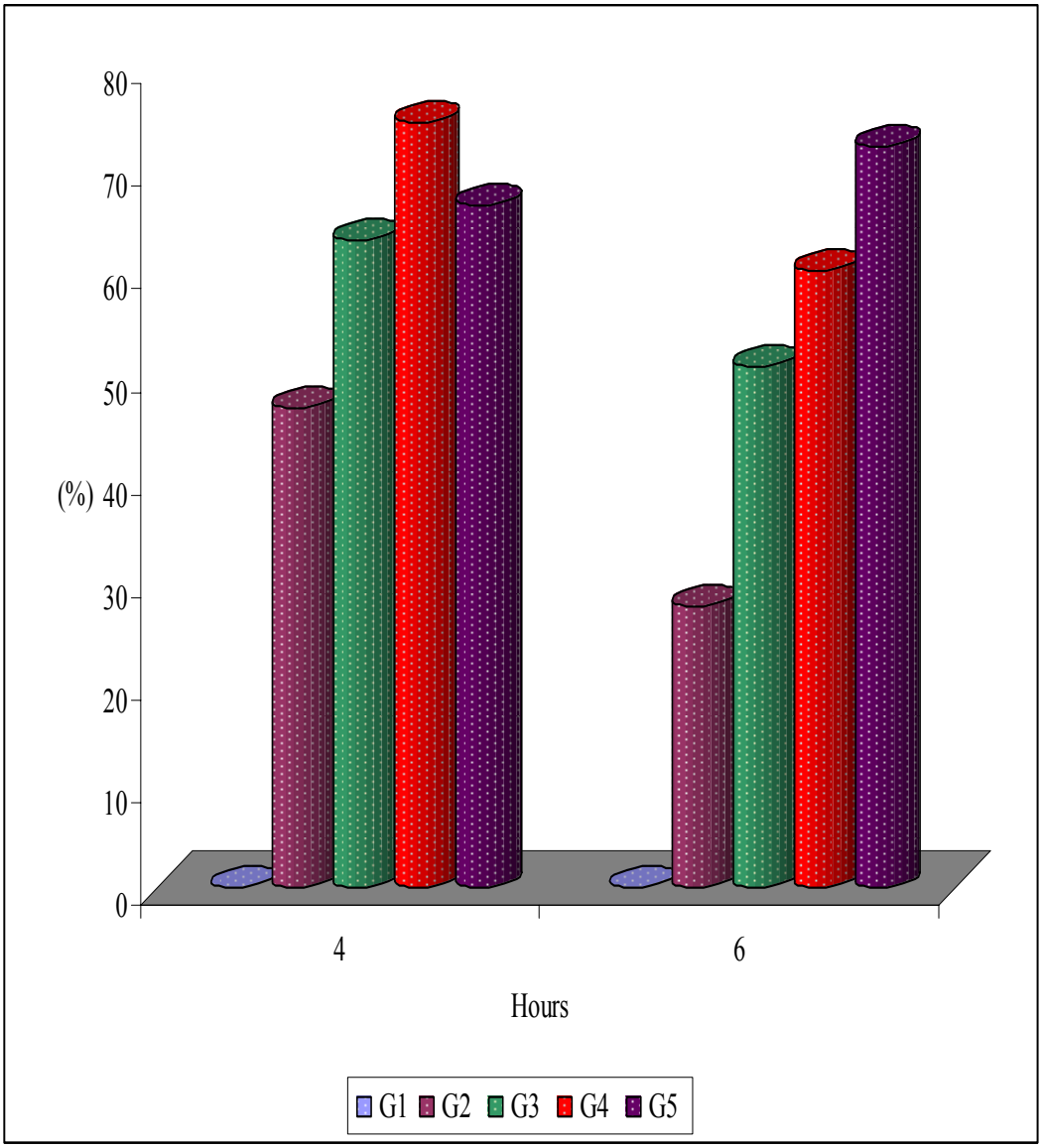
Values are mean ± S.E.M; Means in a column bearing same subscript are similar (not significantly different *P > 0.05*).

H.aby. = Hydnora abyssinica



G1	(un-treated control) saline	+ castor oil
G2	100mg/kg *H.abyssinica*	+ castor oil
G3	200mg/kg *H.abyssinica*	+ castor oil
G4	400mg/kg *H.abyssinica*	+ castor oil
G5	3mg/kg Lopramide	+ castor oil

Fig. (4): Comparison of faeces weight in diarrhoeic rats treated with *Hydnora abyssinica* aqueous extract



G1	(un-treated control) saline	+ castor oil
G2	100mg/kg *H.abyssinica*	+ castor oil
G3	200mg/kg *H.abyssinica*	+ castor oil
G4	400mg/kg *H.abyssinica*	+ castor oil
G5	3mg/kg Lopramide	+ castor oil

Fig. (5): Comparison of inhibition percentage of faeces weight in diarrhoeic rats dosed orally with *Hydnora abyssinica*

At 24 hrs the concentration of total protein and calcium showed increases in all groups while sodium, potassium and albumin demonstrated no significant changes in concentrations.

3. 1. 5 Haematological findings

Table 4 is summarizing the haematological changes in rats blood treated orally with *Hydnora abyssinica* aqueous extract for 24 hours.

In all groups, there is significant increase ($P<0.05$) in values of RBC_s and Hb concentration when compared with the control group. All groups showed significant decrease ($P<0.05$) in PCV and MCHC while values of MCV demonstrated no changes when compared with the control group.

3. 2 Response of Albino rats to *Hydnora abyssinica* aqueous extract

3. 2. 1 Clinical changes

Rats in groups 2 (400mg/kg), 3 (800mg/kg) and 4 (1600mg/kg) showed no signs of toxicity during the experimental period (7 days) and no mortalities were recorded. Also, no abnormal behavior was observed in the un-dosed control rats (group 1) and no deaths too.

3. 2. 4 Changes in serum metabolites

Table 5 and **Table 6** demonstrate the changes in serum metabolites of rats treated with *Hydnora abyssinica* aqueous extract for 7 days.

The activities of AST and ALT showed no significant changes ($P>0.05$) for all groups at day zero. Also concentrations of sodium, potassium, total protein and albumin of the test groups demonstrated no significant ($P>0.05$) changes from the control group. No abnormal values were recorded in the control group at day zero.

Table 2. Values of serum metabolites of orally treated rats with *Hydnora abyssinica* roots aqueous extract at 4 hours

Groups/dose	Total Protein g/dl	Albumin g/dl	Sodium mg/dl	Potassium mg/dl	Calcium mg/dl
G1 un-treated control (saline) + castor oil	02.24 ± 00.17**c**	01.76 ± 00.21 **b**	106.30 ± 01.93**c**	00.98 ± 00.07 **c**	14.18 ± 00.20 **b**
G2 100mg/kg *H.abyssinica* + castor oil	02.70 ± 00.12**c**	02.00 ±00.18**ab**	106.50 ± 01.42**c**	01.24 ±00.09**ab**	14.10 ± 00.15**bc**
G3 200mg/kg *H.abyssinica* + castor oil	03.22 ± 00.15**b**	02.26 ±00.07**ab**	117.78 ±01.42**b**	01.44 ±00.15**ab**	14.06 ±00.16 **bc**
G4 400mg/kg *H.abyssinica* + castor oil	04.52 ± 00.16**a**	02.50 ± 00.17 **a**	139.26 ±00.67**a**	01.74 ± 00.12 **a**	13.64 ± 00.10 **c**
G5 3mg/kg loperamide +castor oil	04.32 ± 00.19**a**	02.50 ± 00.15 **a**	137.30 ±00.46**a**	01.78 ± 00.11 **a**	12.76 ± 00.21 **d**

Values are average (mean ± S.E.M.).

Means in a column bearing same subscript are similar (not significantly different *P > 0.05*).

Table 3. Values of serum metabolites of treated rats with *Hydnora abyssinica* aqueous extract at 24 hours.

Groups/dose	Total protein g/dl	Albumin g/dl	Sodium mg/dl	Potassium mg/dl	Calcium mg/dl
G1 un-treated control (saline) + castor oil	04.96 ± 00.27 **b**	03.46 ± 00.22 **a**	134.80 ± 02.07 **ab**	05.28 ± 00.19 **b**	11.56 ± 00.13 **c**
G2 100mg/kg *H.abyssinica* + castor oil	06.00 ± 00.19 **a**	03.10 ± 00.10 **ab**	135.50 ± 01.65 **a**	05.68 ± 00.21 **ab**	12.46 ± 00.18 **b**
G3 200mg/kg *H.abyssinica* + castor oil	06.16 ± 00.25 **a**	03.18 ± 00.14 **ab**	131.76 ± 00.99 **b**	05.80 ± 00.30 **ab**	12.40 ± 00.16 **b**
G4 400mg/kg *H.abyssinica* + castor oil	05.98 ± 00.16 **a**	03.00 ± 00.15 **ab**	135.00 ± 00.91 **ab**	06.00 ± 00.22 **ab**	13.60 ± 00.19 **a**
G5 3mg/kg loperamide + castor oil	06.58 ± 00.17 **a**	03.30 ± 00.14 **a**	132.26 ± 00.81 **ab**	05.70 ± 00.20 **ab**	12.68 ± 00.14 **b**

Values are average (mean ± S.E.M.).

Means in a column bearing same subscript are similar (not significantly different *P > 0.05*).

Table 4. Haematological values of rats treated with *Hydnora abyssinica* aqueous extract at 24 hours.

Groups/dose	$RBC_S.10^6/mm^3$	Hb (g/dl)	PCV%	MCV(*fl*)	MCHC%
G1 un-treated control (saline) + castor oil	05.21 ± 00.17 **b**	10.48 ± 00.19**b**	39.54 ± 00.28 **a**	64.06 ± 00.26 **ab**	35.54 ± 00.22 **a**
G2 100mg/kg *H.abyssinica* + castor oil	05.49 ± 00.10 **a**	11.52 ± 00.15 **a**	37.16 ± 00.61 **b**	62.96 ± 01.35 **b**	33.88 ± 00.26 **b**
G3 200mg/kg *H.abyssinica* + castor oil	05.52 ± 00.19 **a**	11.58 ± 00.29 **a**	33.76 ± 00.50 **c**	63.24 ± 00.39 **b**	34.30 ± 00.34 **b**
G4 400mg/kg *H.abyssinica* + castor oil	05.43 ± 00.18 **a**	12.08 ± 00.18 **a**	32.08 ± 00.16 **d**	65.38 ± 00.47 **a**	33.50 ± 00.33 **b**
G5 3mg/kg loperamide +castor oil	05.56 ± 00.16 **a**	11.92 ± 00.97 **a**	33.70 ± 00.31 **c**	63.82 ± 00.94 **b**	33.64 ± 00.19 **b**

Values are average (mean ± S.E.M.).

Means in a column bearing same subscript are similar (not significantly different *P > 0.05*).

At day 8, the values of serum ALT, total protein and albumin of the tested groups showed no significant changes ($P<0.05$) when compared with the control group while the concentrations of AST, sodium and potassium showed significant ($P<0.05$) increase in groups 3 and 4. Normal values were recorded for the control group.

3. 2. 5 Haematological findings

Table 7 and **Table 8** indicate the haematological changes in blood of rats dosed orally with *Hydnora abyssinica* roots aqueous extract for 7 days.

The RBC_s, Hb, PCV, MCV and MCHC concentrations represent no significant changes for all groups at day zero. No abnormal values were recorded from the control group.

At day 8, treatment effect showed significant ($P<0.05$) decrease in the number of RBC_s, concentrations of Hb and PCV values. MCV for all groups showed no significant ($P>0.05$) change when compared with the control group while MCHC demonstrated significant increase. Normal values were recorded for the control group.

Table 5. Values of serum metabolites of rats dosed orally with *Hydnora abyssinica* aqueous extract at day zero

Groups/dose	Sodium Mg/dl	Potassium mg/dl	Total Protein g/dl	Albumin g/dl	ALT (i.u/l)	AST (i.u/l)
G_1un-dosed control	146.40±01.24**a**	05.08±00.29 **a**	07.37±00.13 **a**	04.30±00.13 **a**	36.60±00.40 **a**	126.60±01.29**a**
G_2 400mg/kg *H.aby*ssinica	146.47±01.11**a**	05.02±00.16 **a**	07.23±00.19 **a**	04.26±00.11 **a**	36.00±00.63 **a**	128.40±02.50**a**
G_3 800mg/kg *H.aby*ssinica	146.53±01.08**a**	04.92±00.11 **a**	07.31±00.13 **a**	04.32±00.10 **a**	37.00±01.63 **a**	129.00±02.43**a**
G_4 1600mg/kg *H.aby*ssinica	146.60±00.87**a**	05.06±00.17 **a**	07.40±00.11 **a**	04.30±00.17 **a**	37.60±00.40 **a**	127.60±01.78**a**

Values are average (mean ± S.E.M.).

Means in a column bearing same subscript are similar (not significantly different *P > 0.05*).

Table 6. Values of serum metabolites of rats dosed orally with *Hydnora abyssinica* aqueous extract at day 8

Groups/dose	Sodium Mg/dl	Potassium mg/dl	Total protein g/dl	Albumin g/dl	ALT (i.u/l)	AST (i.u/l)
G_1un-dosed control	142.40±00.68 **c**	04.80±00.14 **c**	07.56±00.02 **a**	04.20±00.09 **a**	39.60±00.40 **b**	135.00±02.30 **c**
G_2 400mg/kg *H.aby*ssinica	143.20±00.66**c**	04.96±00.13**bc**	07.50±00.20 **a**	04.20±00.09 **a**	39.00±00.45 **b**	136.40±03.30 **c**
G_3 800mg/kg *H.aby*ssinica	144.80±00.58**b**	05.30±00.11**ab**	07.37±00.12**ab**	04.18±00.09 **a**	40.00±01.41 **b**	138.00±03.15**b**
G_4 1600mg/kg *H.aby*ssinica	147.00±00.71**a**	05.40±00.07 **a**	07.46±00.08**ab**	04.34±00.15 **a**	40.60±00.40 **b**	140.30±07.41**a**

Values are average (mean ± S.E.M.).

Means in a column bearing same subscript are similar (not significantly different *P > 0.05*).

Table 7. Haematological values of rats dosed orally with *Hydnora abyssinica* aqueous extract at day zero

Groups/dose	$RBC_S.10^6/mm^3$	Hb (g/dl)	PCV %	MCV (fl)	MCHC %
G_1un-dosed control	09.49±00.15 **a**	14.48±00.05 **a**	35.86±00.12 **a**	52.67±01.00 **a**	33.02±00.43 **a**
G_2 400mg/kg *H.aby*ssinica	09.97±00.10 **a**	14.40±00.16 **a**	35.96±00.17 **a**	52.71±01.02 **a**	33.22±00.49 **a**
G_3 800mg/kg *H.aby*ssinica	09.50±00.17 **a**	14.54±00.13 **a**	35.94±00.26 **a**	52.60±00.87 **a**	33.00±00.45 **a**
$G_4$1600mg/kg *H.aby*ssinica	09.26±00.10 **a**	14.50±00.14 **a**	35.88±00.18 **a**	52.80±01.24 **a**	33.17±00.19 **a**

Values are average (mean ± S.E.M.).

Means in a column bearing same subscript are similar (not significantly different *P > 0.05*).

Table 8. Haematological values of rats dosed orally with *Hydnora abyssinica* aqueous extract at day 8

Groups/dose	$RBC_s.10^6/mm^3$	Hb(g/dl)	PCV%	MCV(fl)	MCHC%
G_1un-dosed control	09.31±00.13 **a**	14.46±00.16 **a**	35.80±00.21 **a**	54.00±01.38 **a**	31.60±00.27 **b**
G_2 400mg/kg *H.aby*ssinica	08.58±00.19 **b**	13.80±00.10 **b**	35.60±00.22 **b**	54.80±00.58 **a**	33.02±00.29 **a**
G_3 800mg/kg *H.aby*ssinica	08.53±00.08 **b**	13.72±00.18 **b**	35.00±00.09 **b**	55.80±00.45 **a**	32.86±00.22 **a**
$G_4$1600mg/kg *H.aby*ssinica	08.55±00.12 **b**	13.90±00.09 **b**	34.90±00.23 **b**	53.20±01.53 **a**	33.30±00.21 **a**

Values are average (mean ± S.E.M.).

Means in a column bearing same subscript are similar (not significantly different *P > 0.05*).

CHAPTER FOUR

DISCUSSION

Diarrhoea is not a disease, but a symptom of some other problems. However, diarrhoea is the most common and economically devasting health problem of farm animals that cause significant losses in both herd and production despite improvements in management practices, prevention and treatment strategies. A study at the U. S. Sheep Experiment Station (Dubois, ID) showed that diarrhoea accounted for 46% of lamb mortality (Schenian, 2009).

In man also acute diarrhoea constitutes a serious problem worldwide. It is a leading cause of morbidity and mortality among children in the developing countries (Coker et al., 1998). However, according to the WHO, (2004) mortality from diarrhoea has declined over the past two decades from an estimated 5 million deaths among children under five to 1.5 million deaths in 2004. Despite these declines, diarrhoea remains the second most common cause of death among children under five globally, following closely behind pneumonia, the leading killer of young children. Together, pneumonia and diarrhoea account for an estimated 40% of all child deaths around the world each year. Nearly one in five child deaths is due to diarrhoea, a loss of about 1.5 million lives each year. The toll is greater than that caused by AIDS, malaria and measles combined. Therefore, the search for new anti-diarrhoeal drugs should still be seen as a fruitful and logical research strategy, hence it is important and useful to identify plants with anti-diarrhoeal activity (Agbor *et al*., 2003) because medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds.

Numerous studies have validated the traditional use of anti-diarrhoeal medicinal plants by investigating the biological activity of their extracts which can act by many different ways; delaying the intestinal transit time, suppressing gut motility, stimulating water adsorption, reducing electrolytes secretion or having anti-spasmodic effects. Tannins and flavonoids, for instance, are some of the phytochemicals present in these extracts and thought to be responsible for anti-diarrhoeal activity by increasing colonic water and electrolyte reabsorption, and so on.

In this study, evaluation of anti-diarrhoeal activity and investigation of toxicity of aqueous root extract of *Hydnora abyssinica* were carried out. The inhibition of experimental diarrhoea and the reduction in faecal output by a substance are the basis of the pharmacological evaluation of a potential anti diarrhoeal agent.

Castor oil causes diarrhoea due to its active metabolite ricinoleic acid which induces permeability changes in mucosal fluid and electrolyte transport that result in hyper secretory response and diarrhoea (Ammon *et al*., 1975 and Gaginella *et al.,* 1975). Ricinoleic acids produces irritation and inflammation of the intestinal mucosa leading to release of prostaglandin-E2 (PG-E2) which markedly increases in portal venous and gut lumen resulting in stimulation of motility and secretion of water and electrolytes into the small intestine (Pierce *et al*., 1971, Beubler *et al*., 1979, Luderer *et al*., 1980). As the précised mechanism of action of castor oil is through elevated prostaglandin biosynthesis hence, inhibition of prostaglandin biosynthesis delayed castor oil induced diarrhoea (Awouters *et al.,* 1978, Bruton, 1985).

Loperamide, which is at present one of the most efficacious and widely employed anti-diarrhoeal drug, was used in this study as a standard anti-diarrhoeal compound. Loperamide effectively antagonized the diarrhoea induced by castor oil (Neimegeer *et al*.1974), prostaglandin (Karim and

Adikan, 1977) or cholera toxin (Farack *et al*., 1981). The therapeutic effect of the Loperamide is believed to be due to its antimotility and antisecretary activity (Couper, 1987).

The results of this study revealed that the aqueous extract of *Hydnora abyssinica* roots against castor oil induced diarrhoea in rats showed highly inhibition percentage in weight of faeces at all doses used (100, 200 and 400mg/kg body wt. /rat) and this inhibition was accompanied by a dose dependent decrease. An interesting observation finding in this extract is that the minimum dose (100mg/kg) of the extract was able to inhibit castor oil-induced diarrhoea from the earliest hours and this may be due to sufficient absorption of the extract beside that the extract may contain potent anti-diarrhoeal ingredients. Moreover, the maximum dose of the extract (400mg/kg) showed almost similar anti-diarrhoeal activity as that of Loperamide (3mg/kg) after 6 hours and even better than the reference compound Loperamide at 4 hours. The inhibitory effect of the plant extract justified the use of the plant as a non-specific anti-diarrhoeal agent in folkloric medicine.

Furthermore, our findings are in agreement with the anti-diarrhoeal activities reported in other medicinal plants against diarrhoea in rats. In a similar study, Sini *et al.* (2008) evaluated the anti-diarrhoeal activity of the aqueous extract of *Combretu sericeium* roots, which was previously reported by Abdulahi *et al.* (2003) as traditional treatment of diarrhoea in West Africa, against castor oil induced diarrhoea in rats and found that the extract (25 and 50mg/kg body wt.) causes a dose dependent protection against castor oil induced diarrhoea. A phytochemical screening of the extract revealed the presence of tannins, flavonoids, glycosides, anthraquinones and alkaloids. Viswanatha *et al.,* (2007) reported inhibition of weight of the induced diarrhoea by plants known as *Thespesia populenea*.

Also, the pretreatment of mice with *Stereospermum kuthianum* aqueous extract (100, 200 and 400mg/kg body wt.) caused a dose-dependent and significant ($P<0.05$) delay in the onset of diarrhoea, frequency of stooling, decreased weight of wet stools and the general diarrhoeal score in mice and this is a possible reason for its anti-diarrhoeal use in traditional medicine. The extract also produced no mortalities in mice at a maximum oral dose of 8g/kg; it is there well tolerated and relatively safe by oral route (Ching *et al.,* 2008).

In this study, also the daily doses of aqueous extract of *Hydnora abyssinica* roots administrated orally to rats caused neither death nor any observable clinical toxicity signs in rats during the experimental period (seven days) at the doses used. Even a dose as high as 1600mg/kg body wt/rat/day, showed neither death nor any untoward clinical signs. Hence the extract was found to be safe and well tolerated till 1600mg/kg body wt.

The dose (1600mg/kg) equals sixteen times the least extract dose (100mg/kg) that showed significant inhibition in weight of the induced-diarrhoea and this supports the suggestion that the extract has a wide range of safety and its administration may not cause immediate toxic effect at least at the doses used in this study.

Viswanatha *et al.,* (2007) studied the acute toxicity of the aqueous and alcoholic extracts of stem barks of *Thespesia populenea* (Malvaceae). Studies revealed that both the extracts are safe up to 2000mg/kg. The extracts also showed significant dose dependent reduction of cumulative wet mass when investigated for their anti-diarrhoeal activity on castor oil induced diarrhoea model in rodents. However, it was observed that aqueous extract is having more potent anti-diarrhoeal activity than alcoholic one.

The activities of enzymes AST and ALT in the serum were used routinely to assess the functional status of the liver in both clinical and experimental settings (Yanpellewar *et al.,* 2002). In our study we observed that there was no change in the concentrations of ALT, while in the AST values there were increase in the doses 800mg/kg and 1600mg/kg and this may be due to highly doses but not indicated toxicity because not only the increase in serum levels of these hepatic markers but also in billuribin beside the correlated histopathlogical changes in the liver can assess hepatic damage.

On the other hand, the increase in the total protein and albumin was a positive indicator that routinely used for the assessment of the renal toxicity (Ahmed and Adam, 1979 and Medani, 2003). The present study showed that there was no increase in the concentration of total protein and albumin when compared to the un-dosed control group at the end of the experiment and that indicates the safety of the plant. On the other hand, sodium and potassium showed at doses 800 and 1600mg/kg body wt. increase in concentrations.

In this study, haematological values of rats dosed orally with *Hydnora abyssinica* decrease in the number of RBC_S, concentration of Hb and PCV. MCV showed no significant while values of MCHC increased in all groups.

The results obtained in this study showed that the extract significantly reduced the weight of faeces of the induced diarrhoea in rats indicate the anti-diarrhoeal activity of the plant suggesting that the active principles are present in the extract may act as anti-diarrhoeal. It is well established that the release of several mediators such as prostaglandins, some plants' constituents can significantly inhibit the biosynthetic pathway of these mediators (Speroni *et al.,* 2005). Based on these observations, it seems that the anti-diarrhoeal effect of *Hydnora*

abyssinica aqueous extract may be due to the inhibition of prostaglandin biosynthesis or by decreasing the peristaltic movement. However, the mechanism of inhibition is unknown since the exact active ingredients responsible for inhibition were not determined. On further characterization and purification of the active ingredients, more potent compounds can be produced.

Conclusion

- The aqueous extract of *Hydnora abyssinica* possesses anti-diarrhoeal activity verified by the highly percentage of inhibition effected by the extract in weight of faeces. We concluded that, the extract is promising an effective anti-diarrhoeal agent and this provide evidence that supports the claim of traditional use of the plant in treating diarrhoea.
- *Hydnora abssinica* aqueous extract showed no signs of toxicity to rats up to 1600mg/kg revealed that the extract is well tolerated and relatively safe by oral route.

Recommendations

- Further detailed investigations on the phytochemical constituents present in this extract are needed to test their anti-diarrhoeal potential and to clarify the mode of action of the extract.
- Testing of other methods of plants' extraction is needed to reveal if one extract is having more potent anti-diarrhoeal effect than others.
- The study has also, demonstrated details of the oral toxicity of *H. abyssnica* aqueous extract to rats for a period of seven days. Studies are needed to investigate this extract for a longer period using higher doses.

REFERENCES

AbdelGadir, B. S. (2004). Isolation and characterization of *ETEC* causing diarrhoea in calves and man. M. V. M thesis University of Khartoum Sudan.

Abdullahi, A. L., Agho, M. O., Amos, S., Gamaniel, K. S. and Wambebe, C., (2001). Anti-diarrhoeal activity of aqueous extract of *Terminalia avicennoides* roots. Phytoter. Res. 15: 431- 434, Nigeria.

Abdullahi, M., Muhammad, G., Abdulkadir, N. U. (2003). Combretaceae. Medicinal and Economic Plants of Nupeland, p.68.

Acres, S. D. Laing, C.J; Sudaners, J. R. (1975). Acute undifferentiated neonatal diarrhoea in beef calves. Occurrence and distribution of infectious agents. Canadian Journal of comparative medicine, 39: 116-132.

Adam, S. E. I. (1978). Toxicity of indigenous plants and agricultural chemicals in farm animals. Clinical Toxicology, 13, 269.

Agbor, G. A.; Léopold, T. and Jeanne, N. Y., (2003). Medicinal Plants and Traditional Medicine Research Centre, Institute of Medical Research and Medicinal Plants Studies, University of Yaounde I, Cameroon.

Ahmed, O. M. M. and Adam, S. E. I. (1979). Toxicity of *Jatropha curcas* in sheep and goats. Research in Veterinary Science, 27, 89-96.

Aiello, S. E. and Mays, A. (1998). The Merck Veterinary Manual. 8th ed. Merck and Co. Inc., Whitehouse Station, N J, USA and Merial Limited.

Akah, P. A. P. A., Aguwa, C. N., Agu, R. U., (1998). Studies on the anti-diarrhoeal properties of *Pentaclethra macrophylla* leaf extracts Department of Pharmacology and Toxicology, University of Nigeria, Nigeria.

Ali, K. H. (2000). Studies on collibacillosis infectious diarrhoea in calves, sheep and goat. M. V. Sc. Thesis, University of Khartoum.

Ammon, H. V., Thomas, P. J. Bass, P. (1975). Effect of oleic acid and ricinoleic on net jejunum water and electrolyte movement. J. Clin. Invest. 53: 374- 379.

Amstutz, H. E. (1965). Occurance and etiology of infectious calf diarrhoea. JAVMA, 147: 1360- 1363.

Anderson, B. C. and Hall, R. F. (1985). Cryptosporidial infection in Idaho diary calves. JAVMA, 181: 484– 485.

Atta, A. H. and Mouneir, S. M. (2004a). Antidiarrhoeal activity of some Egyptian medicinal plant extracts. Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt.

Atta, A. H. and Mouneir, S. M. (2004b). Evaluation of some medicinal plant extracts for anti-diarrhoeal activity Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt.

Awouters, F., Nimegrees, C. J. E., Lanaerts, F. M., Janssen, P. A. J. (1978). Delay of castor oil in rats: A new way to evaluate inhibitors of prostaglandin biosynthesis. J. Pharmacol. 30: 41-5.

Babuik, L. A., Sabara, M. And Hudson, G. R. (1985). Rotavirus and coronavirus infections in animals. Progress Vet. Microbiol. Immun. Vol. 1. (Pandey, R. ed.). Karger, Basel, Switzerland. pp. 80–120.

Bachmann, P. A. (1982). Comparative aspects of pathogenesis and immunity in animals. Virus infection of gastrointestinal tract (D. A. J. Tyrell, A. Z. Kapikian; eds.). Marcel Dekker, Inc., New York, pp. 361-397.

Baljer, G. (1986). Therapy and prophylaxis of diarrhoea in new born calves. Proveterinario, 2: 6-8.

Beentje, H., and Luke, Q. (2002). Flora of Tropical East Africa, page 1.

Behrman, R. E., Kliegman, R. M. and Jenson, S. B. (2004). Nelson's Text Book of Pediatrics. 17th ed. Sadaners, Anim. Print of Elsevoier Science. New York. London.

Benfield, D. A. and Saif, L. J. (1990). Cell culture propagation of corona virus isolated from cows with winter dysentery. Clin. Microbiol., 28 (6):1454-1457.

Besra, S. E., Gomes, A., Chaudhury, L., Vedasiromoni, J. R. and Ganguly, D. K. (2000). Anti-diarrhoeal activity of seed extract of *Albizzia lebbeck* Benth. Division of Pharmacology and Experimental Therapeutics, Indian Institute of Chemical Biology.

Beubler, E., Juan, H. (1979). Effect of ricinoleic acid and other laxatives on net water flux and prostaglandin E release by the rat colon. J. Pharmacol. 31: 681-685.

Blood, D. C., Radositis, O. M., Henderson, J. A., Arundel, J. H. and Gray, C. C. (1983). Veterinary Medicine, 6th ed., Bailliere Tindall Pitman Press Ltd. London, England, pp. 557–570/ 767–774.

Boulos, I. (1983). Medicinal plants of North Africa. Reference Publications, Inc. (pub). USA.

Bruton, L. L. (1985). Agents affecting gastrointestinal water flux and motility digestant; and bile acids. In; A. G. Gillman, T.W. Rail, A.S. Nies and P. Taylor, Editors, Pharmacol. Basis of Therapeutics, 8th ed, vol. 2, Mc Graw, NewYork, p. 14.

Castrucci, G., Figeri, F., Angellio, V., Ferrari, M., Cilli, V. and Alidrovandi, V. (1987). Field trail evaluation of an inactivated rotavirus vaccine against neonatal diarrhoea of calves. Eur. J. Epidemiol., 3 (1): 5-9.

Chaves, M. C., Santos, F. A., Menezes, A. M. S. and Rao, V. S. N., (1998). Experimental evaluation of *Myracrodruon urundeuva* barks extract for anti-diarrhoeal activity. Departmento de Fisiologia e Farmacologia, Universidade Federal do Ceará, Centro de Ciências da Saúde, C.P. 3157, 60430-270 Fortaleza, CE, Brazil Per Claeson, Gunnar Samuelsson.

Ching, F. P., Omogbai, E. K. I., Ozolua, R. I. and Okpo, S. O. (2008). Antidiarrhoeal activities of aqueous extract of *Stereospermum kunthianum* stem bark in rodents. Nigeria. African Journal of Biotechnology Vol. 7 (9), PP. 1220- 1225.

Ciba Found Symp. (1994). Ethnopharmacological investigation of Chinese medicinal plants. 185: 169- 73; discussion 173-7. Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing.

Clarke; Myral; Harvey, D. G. and Humphreys, D. J. (1981). Veterinary Toxicology 2ed ed., The English language Society, London.

Coker, M. F., Berky, S., Pandou, C. (1998). New development in acute diarrhoea current problem. Paediatrics. 24: 15-17.

Collins, F. M. (1974). Vaccines and cell mediated immunity. Bacteriol. Rev., 38: 371-401.

Couper, M. (1987). Opioid action on the intestine: The importance of the intestinal mucosa. Life Sci. 41: 917- 925.

Cruickshank, J. R., Duguid, J. P., Marimion, B. P. and Swain, R. H. A. (1973). Medical Microbiology, 12th edition. 11. Churchill Livingstone. London, UK.

Deleeuw, P. W., Ellen, D.J., BalRen jam VAN and Straver, P. J. (1980). Rotavirus infection in cattle. Efficacy of oral vaccination in endemically infected herds. Res. Vet. Sci., 29: 142- 147.

Dickle, C. W., Klinkerman, D. L. and Petrie, R. I. (1978). Enterotoxaemia in two foals. Journal of the American Veterinary Medical Association. 173: 306-307.

Durham, P. J. K. Hassard, L. E., Armstrong, K. R. and Naylor, J. M. (1989). Coronavirus associated diarrhoea (Winter dysentery) in adult cattle. Can. Vet. J., 30 (10): 825- 827.

EL Gazali, G. E. B. ; Abdalla, W. S. ; El egami, A. A. B. ; AL Magboul, A. Z. I. and Daim Hamad, A. A. (2004). Aromatic plants of the Sudan, National Center for Research. Khartoum University press.

Farack, U. M.; Kantz, U. and Loescke, K. (1981). Loperamide reduces the intestinal secretion but not the mucosal cAMP accumulation induced by cholera toxin. Naunyn Schmiedebergs Arch. Pharmacol. 317: 178-179.

Farthing, M. J. G. (2002). Novel targets for the control of secretory diarrhoea. Gut 50(suppl. 111): 15-18.

Fernelius, A. L. (1973). Comments on immunity to Neonatal calf diarrhoea viruses and parvovirus infections in calves. Journal of the American Veterinary Medicine Association. 163: 887–888.

Ferris, D. H. (1971). Epizootiology of porcine transmissible gastroenteritis. Ad. Vet. Sci. Comp. Med., 17: 57–86.

Fleetwood, A. J., Edwards, S. and Foxwell, P. W. (1989). Winter dysentery in adult cattle. Vet. Rec., 125 (22): 533-554.

Gaginella, T. S., Stewart, J. J., Olson, W.A., Bass, P. (1975). Actions of ricinoleic acid and structurally related fatty acids on the gastrointestinal tract II. Effects on water and electrolyte absorption in vitro. J. Pharmacol. Exp. Ther; 195: 355-361.

Galvez, J.; Crespo, M. E.; Zarzuelo, A.; De Witte, P. and Spiessens, C. (1992). Pharmacological activity of a procyanidin isolated from *Sclerocarya birrea* bark: Anti-diarrhoeal activity and effects on isolated guinea-pig ileum. School of Pharmacy, University of Granad, Belgium.

Galvez, J.; Zarzuelo, A.; Crespo, M. E.; Utrilla, M. P.; Jiménez, J.; Spiessens, C. and de Witte, P. (1991). Anti-diarrheic activity of *Sclerocarya birrea* barks extract and its active tannin constituent in rats. Departamento de Farmacologia, Facultad de Farmacia, Universidad de Granada, Granada, Spain. Laboratory of Pharmaceutical Biology and Phytopharmacology, K. U. Leuven, Leuven, Belgium.

Gamal, E. D; Mahgoob, S. EL; Awatif, A. B and Mohammed, G. M. (1997). Medicinal Plant of Ingassana area, Research Institute for Medicinal and Aromatic Plants. National Center for Research, Khartoum, Sudan.

Heyworth, M. E. (1985). Pathophysiology of small intestine. The biological principles of disease. By Smith, L. H. and Their, S. O. 2nd ed.

Saunders, Philadelphia, London, Toronto Mexico city, Rio de Janeiro, Sydney, Tokyo and Hong Kong. P. 1211–1212.

Hornish, M., Salajka, E., Sarmanova, Z., Ulman, L. and Sedlacek, M. (1975). Histopathological changes produced by two enteropathogenic strains of *E. coli* in gnotobiotic piglets. J. Comp. Pathol. 85: 277- 283.

Hyde, M. A. and Wursten, B. (2010). Flora of Zimbabwe: species information: Hydnora abyssinica.

Janke, B. H. (1989). Protecting calves from viral diarrhoea. Symposium on neonatal calf diarrhoea Vet. Med., 84: 803-810.

Jones, R. N., (1996). Impact of changing pathogens and antimicrobial susceptibility patterns in the treatment of serious infection in hospitalized patients. AM. J. Med. 100 (6A): 35-125.

Karim, S. M. M. and Adaikan, P. G. (1997). The effect of loperamide on prstaglandin-diarrhoea in rats and man. Prostaglandins 13: 321-331.

Kasper, D. L., Favcl, A. S., Longo, D. L., Brounwald, E., Hauser, S. L. and Jameson, J. L. (2005). Principles of Internal Medicine. 16th ed. Mc. Craw. Hill. Sydney, New York, London, Philadelphia.

Koko Waro, J. O. (1976). Medicinal plants of East Africa. East Africa literature. Bureau. Kampala, Nairobi, Dar Es Salam.

Komolafe, O. O., Anyabuike. C. P. and Obaseki, A. O. (1988). The possible role of mixed function oxidases in the hepatobiliary toxicity of *Azadirachta indica.* Fitoterapia, LIX: (2), 109-113.

Kumar, S, Deven, S, Sangraula, H, kumar, V.L. (2001). Anti-diarrhoeal activity of latex of Calotropis procera. J. Ethnopharmacol. 76: 115- 118.

Lambert, G. and Fernelius, A. L. (1968). Bovine viral diarrhoea virus and *Escherichia coli* in neonatal calf enteritis, Can. J. comp. Med., 32: 440- 446.

Lambert, H. P. (1979). Clinics in Gastroenterology. W. B. Saunder. London. Phladelphia USA.

Lewis, L. D., Philips, R. W. and Card, C. S. (1973). Pathogenesis and treatment of bovine neonatal diarrhoea. JAVMA, 163 (10): 1189-1193.

Lewis, L. D., Philips, R. W. and Elliot, E. D. (1975). Change in plasma glucose and lactose concentration and enzyme activities in the neonatal calf with diarrhoea. Am. J. Vet. Res., 36: 413-416.

Logan, E. F. (1973). Studies on the immunity of the calf to enteric colibacillosis. Fellowship thesis, Royal College of veterinary Surgeons, London, SWI, 39 pp.

Logan, E. F. (1974). Colostral Immunity to Colibacillosis in neonatal calf. British Veterinary Journal. 103: 405.

Lolekha, S. (1986). Consequence of treatment of gastrointestinal infection. Scand. J. Infec. Dis. (supp), 49:154-159.

Luderer, J. R., Dermers, I. M., Hayes, A. T. (1980). Advance in prostaglandin and thromboxane research. New York: Raven Press.

Martin, R. K. (1989). Diarrhoea. Text Book of Veterinary internal Medicine disease of the dog and cat vol. Third edition. Ettinger, S. J. W. B. Saunders Company.

McBeath, I. (1971). An examination of the influence of husbandry on the plasma immunoglobulin in level of the newborn calf using a rapid refractometer test for assessing immunoglobulin content. Vet. Rec., 88: 266.

McDonnell, G. and Russel, A. D. (1999). Antiseptic and disinfection activity, action and resistance. Clinical microbiology reviews 12, 147-79.

Mebus, C. A. (1976). Calf diarrhoea induced by corona and reovirus–like agent. Modern vet. Practice, 57 (9): 693–698.

Mebus, C. A., Stair, E. L., Underdahl**, N. R. and Twiehaus, M. J. (1971).** Pathology of neonatal calf diarrhoea induced by reo–like virus. Vet. Path. 8: 490– 505.

Meckes, M., Torres, J., Calzada, F., Rivera, J., Camorlinga, M., Lemus, H. and Rodríguez, G., (1996). Antibacterial Properties of *Helianthemum glomeratum*, a Plant Used in Maya Traditional Medicine to Treat Diarrhoea. Programa de Colaboración sobre Medicina Indígena Traditionally Herbolaria. Centro de Investigations Ecológicas del Sureste, San Cristóbal de las Casas, Edo. De Chiapas, México.

Medani, A. B. (2003). Toxicity of Poly Damac (Polydiallyl-dimethyl Ammonium Chloride) and Alum (Aluminum Sulphate) to Nubian goats and New Zealand rabbits. Ph.D, Thesis, University of Khartoum, Sudan.

Medterms dictionary (2007). Definition of Diarrhoea. Medterms.com. http://www.medterms.com.

Moore, D. A. (1989). Minimizing morbidity and motility from cryptosporidiosis. Vet. Med. 84, 811–815.

Morin, M.; Lariviere, S.; Lallier, R.; Begin, M.; Roy, R. and Ethier, R. (1978). Neonatal calf diarrhoea: Pathology and microbiology of spontaneous cases in diary herd and incidence of the enteropathogens implicated as etiological agents. Proceeding second International symposium on neonatal diarrhoea. October 3-5. University Saskatchewan. Canada. P 347- 369.

Murugesan, T., Ghosh, L., Mukherjee, K., Das, J., Pal, M. and Saha, B. P., (1999). Evaluation of anti-diarrhoeal profile of *Jussiaea suffruticosa* Linn. Extract in rats Calcutta, India.

Musselman, L. J. (1997). *Hydnora abyssinica* A.Braun ex Schweinf. (Family Hydnoraceae). FZ, Vol 9 Part 2, page 16.

Newman, L. E., Whitehair, C. K. and Mullaney; T. P. (1978). Immune response of the bovine fetus to in-utero vaccination with bovine coronavirus. In ACRES, Proc. 2nd . Int. Symp. Neonatal Diarrhoea. Pp. 457-464.

Niemegeers, C. L. E.; Lenaerts, F. M. and Janseen, P. A. J. (1974). Loperamide (R- 18553), A novel type of anti-diarrhoeal agent. Part 1: *in vitro* oral pharmacology and acute toxicity. Comparison with morphine, codeine, diphenoxylate and difinoxine. Arzneimittelfforsch 24: 1633-613.

Nwude, N. (1979). Poisonous Plants in Nigeria, PhD. Thesis, Ahmadu Bello University Press, Zaria, Nigeria.

Ojewole, J. A. O.; Awe, E. O. and Nyinawumuntu, A. (2008). Antidiarrhoeal activity of *Hypoxis hemerocallidea* Fisch. & C. A. Mey. (Hypoxidaceae) Corm ("African potato") aqueous extract in rodents. South Africa.

Ott, G. L. (1937). Studies on the relationships between colon bacilli and the protozoan parasite, Eimeria tenella, of chickens. A Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

Patel, N. J.; Gujarati, V, B.; Gouda, T. S.; Venkat Rao, N.; Nandakumar, K.; Shantakumar, S. M. (2006). Anti-diarrhoeal activity of alcoholic and aqueous extracts of roots of *Tylophora indica* (Wight and Arn.) in Rodents. Pharmacology on line 1: 19-29.

Pierce, N. F., Carpenter, C. C. J., Elliot, H. Z., Greenough, W. B. (1971). Effects of protaglandins, theophylline and cholera exotoxin upon transmucosal water and electrolyte movement in canine jejunum. Gastroenterlogy; 60: 22-32.

Pohlenz, J. (1980). Bovine cryptosporidiosis. Egyptian Seminar on the mortality of newly born calves. Assuit Vet. Med. J., Vol. 7, supplement 1: 37-41.

Pohlenz, J., Moon, H. W., Cheville, N. F. and Bermick, W. J. (1978). Cryptosporidiosis as a probable factor in neonatal diarrhoea of calves. JAVMA, 172 (4): 452–457.

Puent, J. L. and Finlay, B. B. (2001). Pathogenic *E. coli*. In principle of Bacterial pathogen. Academic Press. P 387−429.

Quinn, P. J., Carter, M. E., Markey, B. K. and Cater, G. R. (1999). Clinical Veterinary Microbiology Mosby.

Radostits, O. M.; Blood, D. C. and Gay, C. C. (1994). Veterinary Medicine. 8th ed. Educational Low-price Books Scheme (ELBS) with Baillière Tindall, London.

Radostits, O. M.; Gay, C. C.; Blood, D. C. and Hinchcliff, K. W. (2000). Veterinary Medicine, A text book of the Diseases of Cattle, Sheep, Pigs, Goats and Horses 9th ed. Harcourt Publishers Limited, London.

Rani, S., Ahmed, N. and Rajaram, S. (1999). Anti-diarrhoeal evaluation of *Clerodendrum phlomidis linn.* Leaf extract in rats. J. Ethnopharmacol , 68: 315-9.

Rhodes, M. B., Stair, E. L., McCullough, R. A., McGill, L. O. and Mebus, C. A. (1979). Comparison of results using electron microscope, immunodiffusion and fluorescent antibody analysis to detect rotavirus in diarrheic faecal samples of calves, Can. J. Comp, Med., 43:84–89.

Rukangira, E. (2001). The African herbal industry constrains and challenges. The natural products and cosmeceuticals conference. Erborisleria Domani (pub).

Russel, A. D. (1999). Bacterial Resistance to disinfectants: Present knowledge and future problem. Journal of hospital infection 43 (suppl.), S57-S68.

Saif, L. J. and Smith, K. L. (1983). A review of rotavirus immunization of cows and passive protection of calves In ACRES, 4th Int. Symp.

Sanders, D. E. (1985). Field management of neonatal diarrhoea. Vet. Clin. N. Amer. Food Anim. Pract., 1 (3): 621- 637.

SAS (1998). SAS Institute, NC, USA.

Schalm, O. W. (1965). Veterinary Hematology. Baillière, Tindall and Cassell Ltd., London.

Schoenian, S. (2009). Sheep and Goat Specialist at the University of Maryland's Western Maryland Research and Education Center. Diarrhoea (scours) in small ruminants. University of Maryland Extension.

Shah, A. J., Gowani, S. A., Zuberi, A. J., Ghayur, M. N., Gilani, A. H., (2009). Anti-diarrhoeal and spasmolytic activities of *Alstonia scholaris* L. Drug Discovery and Natural Products Research Unit, Karachi-74800, Pakistan.

Sini, J. M., Umar, I. A., Anigo, K. M., Stantcheva, I., Bage, E. N. and Mohammed, R. (2008). Antidiarrhoeal activity of aqueous extract of *Combretum sericeum* roots in rats. African journal of Biotechnology Vol.7 (17), pp. 3134-3137.

Smith, H. W. and Hall, S. S. (1967). Observations by the ligated intestinal segment and oral inoculation in pigs, calves, lambs and rabbit; studies on *Escherichia coli* entertoxin. J. Path. Bact., 93: 499-529.

Snedecor, G. W. and Cochran, W. G. (1989). Statistical Methods, 8th ed., Iowa State University press, Iowa, USA.

Snodgrass, D. R. (1981). Report to cattle project, Tripoli, Libya, on calf morality problems experienced on project farms. Anim. Dis. Res. Associ.

Snodgrass, D. R. (1984). Bovine rotavirus serotypes and their significance for immunization J. Clin. Microbiol., 20: 342-346.

Snodgrass, D. R. and Wells, P.W. (1978a). Passive immunity in rotaviral infection. JAVMA: 173, 5 (2): 565-568.

Snodgrass, D. R. and Wells, P.W. (1978b). The immunoprophylaxis of rotavirus infections in lambs. Vet. Rec., 102: 146-148.

Snodgrass, D. R., Fahey, K. J., Wells, P. W., Campbell, I. and Whitelaw, A. (1980). Passive immunity in calf rotavirus infections; Maternal vaccination increases and prolong immunoglobulin GI antibody secretion in milk. Infect. Immun., 38: 344-349.

Snodgrass, D. R., Stewart, J., Taylor, J., Krauuti, F. L. (1982). Diarrhoea in diary calves reproduced by feeding colostrum from cows vaccinated with rotavirus. Res. Vet. Sci., 82 (1): 70-73.

Stafseth, H. J. (1931). Studies on the pathology of avian coccidiosis. J. Amer. Vet. Med. Assoc. 78: 793–816.

Straits Times, (2007). Diarrhoea kills 3 times more http://www.straitstimes.com.

Tennant, B., Harold, D. and Reina Guerra, M. (1972). Physiological and method factors in the pathogenesis of neonatal enteric infections in calves. JAVMA, 161: 993-1007.

Tyzzer, E. E. (1937). A discussion of gallinaceous birds. Amer. J .Hyg. 10: 269–383.

Tzipori, S. (1981). The aetiology and diagnosis of calf diarrhoea. Veterinary record. 108: 510- 514.

Tzipori, S.; Withers, M.; Robins-Drowne, R; Ward, K. L. and Hayes, J. (1984). Attachment of *E. coli* bearing K88 antigen to equine brush border membranes. Veterinary microbiology 9: 561-570.

Umoh, J. U. (1982). Relative survival of calves in a University herd in Zaria, Nigeria. Br. Vet. J., 138: 507-514.

Varley, H. (1967). Practical Clinical Biochemistry. 4th ed., William Heinemann Medical Books, Ltd., and Interscience Books Inc., New York.

Viswanatha, G. L., Srinath, R., Nandakumar, H., Shylaja, and Lakshman, K. (2007). Antidiarrhoeal activity of *Thespesia populnea* in rodents. Pharmacology online 3: 222-230.

Wakeen, A. A. and Dansoury, M. S. (1962). Study of calf losses in a herd of Sudanese dairy cattle. Sud. J. Vet. Sci. Anim. Husb. , 3 (2): 125−132.

Waltner-Toews, D., Martin, S. W., Meek, A. H., McMillon, I, and Crouch, C. F. (1985). A field trial to evaluate the efficacy of a combined rotavirus –coronavirus, *E.coli* vaccine in diary cattle. Canad. J. Comp. Med., 49: 1-9.

Weber, D. M. (1976). The diarrhoeal disease and food born illness. In tropical medicine, Hunter G.W., Swartzwelder J.C, Clyde D.F (eds), 5th ed. W.B. sounders Co; Philadelphia.

Wells, P. W., Snodgrass, D. R., Herring, J. A. and Dawson, A. M. (1978). Antibody titers to lamb rotavirus in colostrum and milk of vaccinated ewes. Vet. Rec., 103: 46-48.

WHO (1998). Regulatory Situation of Herbal Medicines, A worldwide Review. World Health Organization, Geneva, Switzerland. http://whqlibdoc.who.int/hq/1998/WHO -TRM-98.1.pdf.

WHO, (1980). *E. coli* Diarrhoea Bulletin of the World Health Organization 58:23.

WHO, (1992). Reading on diarrhoea, student, manual WHO the management and prevention of diarrhoea.

Wikipedia, the free encyclopedia, (2007). Manila Bulletin Publishing: Diarrhoea causes 1.5 million infant deaths a year__ UN. NIH Publication No.07- 2749.

Wise, C. M., Knight, A. P., Iwias, M. J., Morris, C. J., Ellis, R. P. and Philips, R. W. (1983). Effect of salicylates on intestinal secretion of calves. AM. J. Vet. Rec., 44 (12): 2221-2225.

Wondergen, P., Senah, K. A., Glover, E. K. (1989). Herbal Drugs in Primary Healthcare. Zimbabwe Science News.

Woode, G. N. and Dennis, M. J. (1978). Studies on cross protection induced in calves by rotavirus of calves, children and foals. Vet. Rec., 103: 32–34.

Woode, G. N. and Bridger, J. C. (1975). Viral enteritis of calves. Vet. Rec., 96: 85-88.

Woode, G. N. and Crouch, C. F. (1978). Naturally occurring and experimentally induced rotaviral infection of domestic and laboratory animals. JAVMA, 123; 5 (2): 522−526.

Yanpellewar, S. U.; Sen, S.; Tapas, S.; Mohan, K. M.; Raju, S. S. and Acharya, S. B. (2002). Effect of *Azadirachta indica* on paracetamol-induced hepatic damage in albino rats. Phytomed., 10: 391-396.

Zeman, D. H., Thomson, T. U. and Francis, D. H. (1989). Diagnosis, treatment and management of enteric collibacillosis. Vet. Med. Vol. 84, 794- 802.